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PATENT APPLICATION

INTERVENTIONS TO MIMIC THE EFFECTS OF CALORIE RESTRICTION

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INTERVENTIONS TO MIMIC THE EFFECTS OF CALORIE RESTRICTION

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] NOT APPLICABLE

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] NOT APPLICABLE

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK.

[0003] NOT APPLICABLE

Field of the Invention

[0004] For years, researchers have attempted to identify biomarkers of aging to facilitate the identification of interventions that might slow or reverse the aging process. Dietary calorie restriction (CR) is the only well-documented method for extending life span in homeothermic vertebrates, and is the most effective means known for reducing cancer incidence. Although many of the physiological consequences of CR were described 65 years ago, there is no consensus regarding its mode of action. Consequently, there has been no practical method o f identifying interventions that might mimic such calorie restriction effects. Rather, a researcher would have to wait the test animal's lifetime to determine whether a particular intervention impacted lifespan and/or cancer incidence.

Description of the Related Art

[0005] Mammals seem to share a common set of genes, and yet they have widely differing life spans. It is impossible to know at present whether the differences in life spans are due to differences in the sequence of specific genes, or to differences in their expression. However, it is clear from many years of study in dozens of laboratories that long term reduction in dietary calorie consumption (CR) delays most age-related physiological changes, and extends life span in all species tested, provided malnutrition is avoided (Weindruch, et al., *The Retardation of Aging and Disease by Dietary Restriction* (Charles C. Thomas, Springfield, Il, 1988)). These studies also have shown that CR is the most effective means now known for

reducing cancer incidence and increasing the mean age of onset of age related diseases and tumors in homeothermic vertebrates (Weindruch et al. (1982) Science 215: 1415). Thus, it seems clear that life spans can be extended through a relatively simple dietary regimen. However, there are no studies on the effects of short-term calorie restriction on metabolism and gene expression.

[0006] One report has been published of gene expression profiling in muscle (Lee et al. (1999) Science 285: 1390). In these studies, many age related changes in muscle gene expression appeared to be prevented or reversed by CR. The expression profiles of 6500 genes were compared among old, long term CR and control mice, and young control mice. Some age-related changes in muscle gene expression appeared to be wholly or partially prevented by CR.

BACKGROUND OF THE INVENTION

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention contemplates a method of identifying interventions within a short time frame that mimic the effects of calorie restriction. Such interventions will lead to increased life span, reduce cancer incidence, and/or increase the age of onset of age related diseases and tumors.

[0008] In a preferred embodiment a method of identifying an intervention that mimics the effects of caloric restriction in cells is disclosed, comprising the steps of:

obtaining a biological sample;

exposing said biological sample to an intervention;

waiting a specified period of time;

assessing changes in gene expression levels, levels of RNA, protein, or protein activity levels related to one or more biomarkers of aging; and

identifying said intervention as one that mimics the effects of caloric restriction if one or more changes in said levels also occurs in caloric restriction.

[0009] The biological sample may be either in vitro or in vivo. In a preferred embodiment, the biological sample comprises cells. In a more preferred embodiment, the cells are obtained from a mammal. In an even more preferred embodiment, the mammal is a mouse.

[0010] In one embodiment, the change in gene expression levels, levels of RNA, protein, or protein activity levels corresponds to a change in gene expression for a gene encoding a chaperone protein. In a preferred embodiment, the chaperone protein is GRP78.

[0011] In one embodiment, said biomarker is apoptosis. In another preferred embodiment, said biomarker is aging. In another preferred embodiment, said biomarker of aging is a production of cancer cells.

[0012] In a preferred embodiment, the changes in said gene expression level, levels of RNA, protein, or protein activity levels related to one or more biomarkers of aging occur in 6 weeks or less. In a more preferred embodiment, the changes in said gene expression levels, levels of RNA, protein, or protein activity levels related to one or more biomarkers of aging occur in four weeks or less. In an even more preferred embodiment, the changes in said gene expression levels, levels of RNA, protein, or protein activity levels related to one or more biomarkers of aging occur in two weeks or less. In a most preferred embodiment, the changes in said gene expression levels, levels of RNA, protein, or protein activity levels related to one or more biomarkers of aging occur in about two days or less.

[0013] In a one embodiment, changes in gene expression are evaluated using a gene chip. In a preferred embodiment, the gene chip contains genes for immune system activation. In another preferred embodiment, the gene chip contains genes for DNA repair. In another preferred embodiment, the gene chip contains genes associated with apoptosis. In another preferred embodiment, the gene chip contains genes for the enteric nervous system.

[0014] In an alternate embodiment, the biological sample is a test animal. In a preferred embodiment the disclosed method additionally comprises determining changes in said levels in a reference animal having identifying characteristics of along term calorie-restricted animal wherein the reference animal has been on a calorie restricted diet for less than about 6 weeks and wherein said changes are used in said identifying said intervention as one that mimics the effects of calorie restriction. In a more preferred embodiment, the reference animal has been on a calorie restricted diet for less than about 4 weeks. In an even more preferred embodiment, the reference animal has been on a calorie restricted diet for less than about 2 weeks.

[0015] In a preferred embodiment, the test animal is a mouse. In a preferred embodiment, changes in gene expression are assessed in the test animal.

[0016] In a more preferred embodiment, the disclosed method further comprises:

obtaining a gene expression profile from a calorie restricted reference animal;

comparing changes in gene expression for the test animal to the gene expression profile of the calorie restricted reference animal; and

identifying said intervention as one that mimics the effects of calorie restriction if the gene expression profile of the test animal is statistically similar to the gene expression profile of the calorie restricted animal.

[0017] In a more preferred embodiment, the gene expression profile of the test animal is determined to be statistically similar to the gene expression of the calorie restricted animal by one way ANOVA followed by Fisher's test ($P<0.05$).

[0018] In another aspect of the invention, a system is disclosed for identifying an intervention that mimics the effects of calorie restriction in a test animal comprising a test animal and a gene chip comprising genes known to have altered expression during calorie restriction. In a preferred embodiment, the gene chip comprises genes selected from the group consisting of genes for immune system activation, genes for DNA repair, genes associated with apoptosis and genes for the enteric nervous system.

[0019] For purposes of summarizing the invention and the advantages achieved over the prior art, certain objects and advantages of the invention have been described above. Of course, it is to be understood that not necessarily all such objects or advantages may be achieved in accordance with any particular embodiment of the invention. Thus, for example, those skilled in the art will recognize that the invention may be embodied or carried out in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objects or advantages as may be taught or suggested herein.

[0020] Further aspects, features and advantages of this invention will become apparent from the detailed description of the preferred embodiments which follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

[0022] These and other feature of this invention will now be described with reference to the drawings of preferred embodiments which are intended to illustrate and not to limit the invention.

[0023] FIG. 1. Effects of feeding on hepatic GRP78 and ERp72 mRNA. At 0, 1.5, 5 and 12 h following feeding, 5 mice from each dietary group were killed. Their weights after 24 h of fasting were 22.96 ± 1.49 for CR and 37.12 ± 1.19 g for control mice. GRP78 mRNA (A) and ERp72 mRNA (B) from control (closed circle) and CR (open circle) mice were

quantified using dot blots. RNA loading and transfer were normalized using data obtained from serial probings for 18S ribosomal RNA and S-II mRNA. Similar results were obtained with both control probes. CR and control mice, fed once daily for 30 days, were fasted for 24 hours and killed (n=5, 0 time point) or refed and killed at the times specified (n=5 for each time point). + represents $P < 0.01$ significance of difference between CR and control at each time point. * represents $P < 0.01$ significance of difference from the 0 time point within each dietary group. The 0 and 24 hour times points are the same data set.

[0024] FIG. 2. The gene and tissue specificity of the chaperone feeding response. A, The domain of chaperone genes responsive to feeding was determined by quantifying hepatic chaperone mRNA abundance using RNA from mice fasted for 48 hours (n=6; open bars) or from mice fasted 48 hours, refed and killed 1.5 h later (n=6; filled bars). The mRNAs were quantified by dot blotting and Northern blotting. There was no significant difference in the results obtained with either technique. The dot blotting results are shown. B, Liver, kidney, and muscle GRP78 mRNA from 24 hour fasted mice (n=4), and from 24 hour fasted mice 1.5 hours after feeding (n=5). These data were from different mice than used in panel A. The statistical significance of the results are indicated (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

[0025] FIG. 3. Effects of CR on hepatic pre-mRNA and GRP78 mRNA abundance. A, RNase protection of pre-mRNA and mRNA in CR and control mice. Hepatic RNA was purified from control and CR mice and hybridized with an RNA probe for transcripts spanning the third intron and fourth exon boundary of the GRP78 gene. The precursor mRNA protected a 223 base region of the probe, labeled GRP78 pre-mRNA, while the GRP78 mRNA protected a 113 base fragment, so labeled in the figure. A probe for S-II mRNA coding sequences was included in each reaction as an internal control. It protected a 185 base fragment labeled S-II mRNA in the figure. Lane 1 shows the protected fragments produced by the GRP78 probe and mouse liver RNA. Lane 2 shows the fragments produced by the S-II probe hybridized to yeast total RNA. Lane 3 shows the results produced by the S-II probe hybridized to mouse liver RNA. Lanes 4, 6, and 8 show the results produced by hepatic RNA from control mice. Lanes 5, 7, and 9 show the results with RNA from CR mice. Quantification of the abundance of the protected fragments representing the GRP78 mRNA (B) and pre-mRNA (C). Studies such as those shown above were conducted using hepatic RNA from 6 CR and 6 control mice. The intensity of the protected fragments was quantified with a phosphorimager. The intensities of the pre-mRNA and mRNA fragments were normalized to the intensity of the protected fragment representing S-II mRNA. Statistical significance is indicated as in the legend to Fig. 2.

[0026] Fig. 4. Effects of feeding on hepatic GRP78 mRNA and pre-mRNA abundance. A, RNase protection of probes for hepatic GRP78 pre-mRNA and mRNA in mice after 48 hours of fasting (n=5), or 1.5 h after feeding of 48 hour fasted mice (n=5). RNA purified from liver was hybridized either to a probe for primary transcripts containing the exon 7 and intron 7 boundary of the GRP78 gene which produced a 257 base protected fragment (labeled S-II + GRP78; lanes 7-12), or to a probe for primary transcripts spanning the exon 7 and intron 7 boundary, which protected a 200 nucleotide fragment (labeled S-II + tGRP78, lanes 13-18), as indicated in the figure. GRP78 mRNA produced a 143 nucleotide fragment representing GRP78 mRNA, as indicated in the figure. A probe for S-II mRNA coding sequences was included in each reaction as an internal control. With this probe, S-II mRNA protected a 277 nucleotide fragment, labeled S-II mRNA in the figure. Lane 1, RNA markers. Lanes 2-6, hybridization of the indicated probes with yeast tRNA. Lanes 7-12, hybridization of the GRP78 and S-II probes with RNA from fasted (lanes 7 9) and refed (lanes 10 12) mice. Lanes 13 18, hybridization of tGRP78 and S-II probes with RNA from fasted (lanes 13-15) and refed (lanes 16-18) mice. Quantification of the abundance of the protected fragments representing the GRP78 mRNA (B) and pre-mRNA (C). Studies such as those shown above were conducted using hepatic RNA from 6 CR and 6 control mice. The intensity of the protected fragments was quantified and normalized as described in Fig. 3 above. Statistical significance is indicated as in the legend to Fig. 2.

[0027] FIG. 5. Effects of protein synthesis inhibitors on the feeding response of GRP78 (A) and PEPCK (B) mRNA. Mice fasted for 48 h were injected i.p. with vehicle and after 1 hour injected a second time i.p with vehicle (Refed+Sham; n=6). Mice fasted for 48 hours were injected i.p. with vehicle 30 min before and 30 min after feeding (Refed+Sham, n=6). Mice fasted for 48 h were injected i.p. with cycloheximide and after 1 hour injected a second time i.p with cycloheximide (Fasted+Cycloheximide; n=6). Mice fasted for 48 h were injected i.p. with cycloheximide 30 min before and 30 min after feeding (Refed+Cycloheximide; n=6). Mice fasted for 48 h were injected i.p. with puromycin and after 1 hour injected a second time i.p with puromycin (Fasted+Puromycin; n=6). Mice fasted for 48 h were injected i.p. with puromycin 30 min before and 30 min after feeding (Refed+Puromycin; n=6). GRP78 and PEPCK mRNA abundance were determined using purified hepatic RNA. Bars without common superscripts are significantly different ($P<0.005$).

[0028] FIG. 6. Regulation of the fasting feeding response by insulin, dibutyryl-cAMP, glucagon, and ingestion of mineral oil and cellulose. A, Groups of six mice were fasted for

48 h and treated as follows: Fasted+Sham mice were injected with vehicle and 1 h later vehicle injected a second time; Fed+Sham mice were sham injected with vehicle 30 min before and 30 min after feeding; Fed+cAMP mice were injected with dibutyryl-cAMP and theophylline 30 min before and 30 min after feeding; Fed+glucagon mice were injected with glucagon 30 min before and 30 min after feeding; Fasted Diabetic+Sham mice, previously rendered diabetic with STZ, were vehicle injected and 1 h later vehicle injected a second time; Fed Diabetic+Sham, STZ diabetic mice were sham injected with vehicle 30 min before and 30 min after feeding; Fed Diabetic+cAMP, diabetic mice were injected with dibutyryl-cAMP and theophylline 30 min before and 30 min after feeding. All mice were killed 1 h after their last injection. Total RNA was isolated from the liver and subjected to dot blot analysis. Bars with no common superscripts are significantly different ($P<0.005$). B, Effects of mineral oil and cellulose ingestion on liver GRP78 mRNA abundance. Groups of six mice were fasted for 48 h and treated as follows: Fasted, mice were fasted for 48 h and killed; Fed, mice were fasted for 48 h, fed, and killed 1.5 h later; Fasted+cellulose, mice fasted for 48 h were fed a mixture of cellulose and mineral oil, and killed 1.5 h later. Significance is indicated as in the legend to Fig. 5.

[0029] FIG. 7. Effects of adrenalectomy and dexamethasone administration on the expression and regulation of hepatic GRP78 mRNA. Groups of six mice were fasted for 48 h and treated as follows: Fasted+Sham, sham operated mice were injected with vehicle IP 7.5 h and 1.5 h before they were killed; Fed+Sham, sham operated mice were injected with vehicle IP 6 hours before and 30 min after feeding, and mice were killed 1 h after the last injection; Adx Fasted+Sham, adrenalectomized mice were injected with vehicle IP 7.5 h and 1.5 h before they were killed; Adx Fed+Sham, adrenalectomized mice were injected with vehicle IP 6 hours before and 30 min after feeding, and the mice killed 1 h later; Adx Fasted+Dex, adrenalectomized mice were injected IP with dexamethasone 7.5 h and 1.5 h before they were killed; Adx Fed+Dex, adrenalectomized mice were injected EP with dexamethasone 6 hours before and 30 min after feeding, and killed 1 h later. Significance is indicated as in the legend to Fig. 5.

[0030] FIG. 8. The hepatic gene expression profiles of old control, old CR, young control, and young CR mice. The mice weighed 37.2+1.9 g, 22.8+1.2 g, 26.0+2.8 g, and 19.4+1.6 g, respectively. The CR groups consumed approximately 50% fewer calories than their control counterparts post weaning, as described. Levels of specific mRNA were determined using the Mu11KsubA and Mu11KsubB GeneChip arrays (Affymetrix, Santa Clara, CA) containing targets for approximately 12,000 known mouse genes and ESTs. The experiment

tree function of GeneSpring 3.0 (Silicon Genetics, San Carlos, CA) was utilized to display the results. The horizontal axis represents the position of each gene assigned by the "gene tree" average linkage hierarchical clustering algorithm of the program. Below the position assigned to each gene is a color coded indication of its relative expression level, based on a continuous scale. Bright blue indicates no detectable expression, purple average expression, and bright red high expression. The average expression of each gene in each group is shown. The GeneSpring "experiment tree" clustering algorithm calculated an average-linkage hierarchical clustering dendrogram of the data for each group of mice, which is shown to the left of the expression profiles.

[0031] FIG. 9. Schematic representation of the hypothesis that CR acts by preventing age related changes in gene expression. During aging, some genes become over expressed or under expressed relative to their levels in young animals (lower and upper lines). Unchanged expression with age is represented by the horizontal line. These deviations are assumed to be deleterious. The important genes effected by CR, in this hypothesis, are the over or under expressed genes returned to youthful levels of expression (arrows). The numbers of genes and ESTs in each category are shown at the ends of the lower and upper lines. The number of known genes in each category returned to baseline expression by LT- and ST-CR are given after the colons. Long-term and short-term CR both acted to reverse or prevent 23 of the increases and 41 of the decreases. Thus, long term LT CR actually prevented the increased expression of only 30 genes and ESTs and the decreased expression of only 24 genes and ESTs.

[0032] FIG. 10. Average of pairwise comparison of the global gene expression correlation coefficient for each possible pair of mice.

[0033] FIG. 11. The hepatic gene expression profiles of young CR, young control and streptozotocin (STZ)-treated mice. Levels of specific mRNA were determined using the Mu11KsubA and Mu11KsubB GeneChip arrays (Affymetrix, Santa Clara, CA) containing targets for approximately 12,000 known mouse genes and ESTs. The experiment tree function of GeneSpring 3.0 (Silicon Genetics, San Carlos, CA) was utilized to display the results. The horizontal axis represents the position of each gene assigned by the "gene tree" average linkage hierarchical clustering algorithm of the program. Below the position assigned to each gene is a color coded indication of its relative expression level, based on a continuous scale. Bright blue indicates no detectable expression, purple average expression, and bright red high expression. The average expression of each gene in each group is shown. The GeneSpring "experiment tree" clustering algorithm calculated an average

linkage hierarchical clustering dendrogram of the data for each group of mice, which is shown to the left of the expression profiles.

[0034] FIG. 12. Average of pairwise comparison of the global gene expression correlation coefficient for each possible pair of mice.

[0035] FIG. 13. The hepatic gene expression profiles of old CR, old control and aminoguanidine (Au) treated mice. Levels of specific mRNA were determined using the Mu11KsubA and Mu11KsubB GeneChip arrays (Affymetrix, Santa Clara, CA) containing targets for approximately 12,000 known mouse genes and ESTs. The experiment tree function of GeneSpring 3.0 (Silicon Genetics, San Carlos, CA) was utilized to display the results. The horizontal axis represents the position of each gene assigned by the "gene tree" average linkage hierarchical clustering algorithm of the program. Below the position assigned to each gene is a color coded indication of its relative expression level, based on a continuous scale. Bright blue indicates no detectable expression, purple average expression, and bright red high expression. The average expression of each gene in each group is shown. The GeneSpring "experiment tree" clustering algorithm calculated an average linkage hierarchical clustering dendrogram of the data for each group of mice, which is shown to the left of the expression profiles.

DETAILED DESCRIPTION OF THE INVENTION

[0036] While the described embodiment represents the preferred embodiment of the present invention, it is to be understood that modifications will occur to those skilled in the art without departing from the spirit of the invention. The scope of the invention is therefore to be determined solely by the appended claims.

[0037] The effects of long term calorie restriction include increases in the rate of clearance of serum proteins, including glucose damaged serum proteins, from the blood as well as changes in gene expression. For example, long term calorie restriction down regulates the expression of certain chaperone genes, up regulates the expression of certain transcription factors and homeobox genes, increases expression of immune system genes, and increases genes enhancing genetic stability and apoptosis. These changes in gene expression correlate with an increase in apoptosis, reduced cancer incidence and increase the turnover of damaged and toxic serum proteins, reducing kidney and vascular damage with age or diabetes.

[0038] Molecular chaperones assist in the biosynthesis, folding, processing, and degradation of proteins. Many of the chaperone genes are stress inducible. Subsets of chaperones are induced by different physiological stressors. For example, the majority of the

known endoplasmic chaperones are induced by stresses that produce malfolded or improperly glycosylated proteins in the ER. This unfolded protein response pathway also may adjust the level of protein trafficking through the ER to the level of ER chaperones. Other chaperones, such as the abundant cytoplasmic chaperone HSC70 are normally thought of as constitutively expressed. The present invention is based in part on the finding that certain chaperone genes are down regulated by calorie restriction (such regulation is thought to be mediated through the insulin and glucagon pathways). The expression of Erp72, Erp57, GRP 170, GRP78, GRP94, HSC70, Calnexin, and Calreticulin are particularly affected by calorie restriction.

[0039] The fasting mRNA and protein levels of nearly every ER chaperone studied were found to be significantly and consistently reduced in the livers of CR mice chronically fed a low calorie diet. In the case of GRP78, levels decreased by approximately 66%. Further, the reduction in chaperone mRNA levels was proportional to the reduction in calorie consumption. The fewer calories consumed, the lower the level of chaperone mRNA. We subsequently found that fasting chaperone mRNA levels changed over the course of 2 weeks in response to different levels of chronic calorie consumption. The more calories consumed per week, the higher the chaperone levels. Chaperone mRNA levels respond more rapidly to calorie consumption.

[0040] mRNA for most ER chaperones, and for the major cytoplasmic chaperone, HSC70, are dynamically responsive (within 1.5 h) to each meal, and to the number of calories consumed. Features of this induction distinguish it from the unfolded protein response. The feeding induction was observed in kidney and muscle tissue, as well as in the liver. Postprandial changes in glucagon, in conjunction with insulin, were found to be the key mediators of this induction.

[0041] Chaperone mRNA abundance responds within 1.5 h to caloric intake. Insulin and glucagon may be important for the response. This feeding response is rapid. By 1.5 hours after feeding, ER chaperone mRNAs were at or near their maximum level of induction. This feeding related induction is not limited to one strain of mouse or to one species. Further, the response is found in tissues other than liver. Thus, it is a response which is generally important to the physiology of a variety of cell types in vivo.

[0042] Because many chaperones are relatively stable proteins, their protein levels change more slowly in response to caloric intake than their mRNAs. For example, GRP78 protein has a half life of over 24 hours in cultured cells. We found that GRP78 protein levels change only over a span of several days in response to changes in average daily calorie consumption. In this way, many chaperones may effectively integrate the rapid mRNA responses to feeding

into longer term changes in chaperone protein levels. Long term differences in average calorie consumption do lead to differences in the hepatic levels of both ER and some cytoplasmic chaperones.

[0043] RNase protection assays indicate that GRP78 mRNA is transcriptionally regulated in response to feeding. Similar RNase protection results were obtained with hepatic RNA from chronically CR mice. Thus, both feeding and CR transcriptionally alter the expression of the chaperone genes.

[0044] Puromycin led to partial induction of GRP78 mRNA. It is unlikely that induction of the mRNA by cycloheximide is due to stabilization of the transcript by polysome aggregation. While cycloheximide protects some mRNAs from inactivation and degradation in this way, puromycin does not. Rather, it inhibits translation by polysome dissociation. Thus, maintenance of low hepatic GRP78 mRNA levels most likely requires the action of an unstable repressor of GRP78 gene expression in fasted mice. In the presence of inhibitors of translation, this repressor may decay, releasing the gene from repression.

[0045] Second, there was no augmentation of GRP78 mRNA induction when feeding and inhibition of translation were combined. While partial induction of the mRNA was found in puromycin treated mice, feeding induced the mRNA to the same level found in the absence of the inhibitor. Further, cycloheximide induced the mRNA to the same extent. Without being bound to any particular mechanism, it is suggested that the inhibitors and feeding may induce the gene through a common pathway.

[0046] Third, since feeding fully induced GRP78 mRNA in puromycin treated mice, de novo protein synthesis is not required for the feeding response. Preexisting signaling and regulatory factors mediate the response. Fourth, the feeding response cannot result from a postprandial increase in protein trafficking through the ER. Enhanced ER de novo protein trafficking can induce chaperone mRNA. However, no such increase could have occurred in the presence of puromycin.

[0047] Fifth, the unfolded protein and growth factor responses are not involved in the induction of chaperones by feeding. Cycloheximide blocks the unfolded protein and growth factor responses. We are aware of only one manipulation besides feeding capable of inducing ER chaperone mRNA in the presence of cycloheximide. GRP mRNAs are induced by cellular hypoxia in culture, and this induction is independent of cycloheximide treatment. Whether the feeding and hypoxia response share common molecular pathways is unknown at present.

[0048] Feeding is well-known to decrease glucagon and increase insulin levels. Both glucagon and dibutyryl-cAMP blunted the feeding induction of GRP78 mRNA. Thus, glucagon is a negative regulator of GRP78 expression in vivo. The feeding induction of GRP78 mRNA was significantly reduced in STZ diabetic mice. Without being bound to any particular mechanism, this result and the absence of a feeding response in STZ-diabetic, dibutyryl-cAMP treated mice indicate that the action of both hormones is required for the response.

[0049] Other effectors which are known to respond to feeding were also examined. Luminal stimuli can promote the release of gastrointestinal hormones. For this reason, we determined whether luminal filling with a non-digestible mixture of mineral oil and cellulose could stimulate chaperone expression. A small but significant response was found. However, insulin and glucagon have a much stronger effect on chaperone mRNAs, indicating they are the signals primarily responsible for the feeding response.

[0050] The feeding response was enhanced in adrenalectomized mice. These results suggest that other adrenal hormones, perhaps catecholamines, may partially blunt the chaperone mRNA response to feeding. However, the mechanism by which these hormones stimulate the feeding response is unknown at present.

[0051] Overall, feeding rapidly and strongly induced the mRNA for the major cytoplasmic chaperone, HSC70, and most ER chaperones examined. Feeding also induced ER chaperone mRNAs in at least three different tissues. Feeding and CR regulated chaperone mRNA abundance at the transcriptional level. Without being bound to any particular mechanism, feeding appeared to release chaperone gene expression from the effects of an unstable inhibitor. Insulin was required, and glucagon and cAMP mediated the feeding response. Postprandial changes in glucagon levels may be the primary mediator of the response. Gastrointestinal and adrenal hormones, but not glucocorticoids also have a role in the feeding response.

[0052] Surprisingly, changes in gene expression are also observed with short-term calorie restriction. These changes in gene expression are virtually identical to the changes observed in long term CR. Short-term calorie restriction occurs when switching a mature test animal to a diet which is about 50% less than a control diet for about 2 6 weeks. In a preferred embodiment, the test animal is a mature mouse and the mature mouse is switched to a calorie restricted diet at about 31 months. Preferably, an intermediate diet which is about 20-40% less than a control diet is employed for about two weeks before switching to a CR diet for an additional two weeks.

[0053] Both long term and short-term CR produces its profound effects on mammalian physiology by affecting the expression of genes. To identify as broadly as possible the effects of caloric restriction on global patterns of gene expression, gene chip technology was utilized to characterize the effects of long and short-term CR on the expression of approximately 11,000 mouse genes in the liver.

[0054] Liver is an attractive organ for study, since it contains a number of cell types, allowing assessment of the effects of CR on hepatocytes, which are primarily responsible for the regulation of metabolism and blood sugar, neurons of the enteric nervous system, immune system cells in the blood, and vascular smooth muscle cells, among others. In liver, by far the predominant effect of caloric restriction is the activation of gene expression. In addition, after only four weeks of caloric restriction, the gene expression profile of old mature mice had been shifted from the profile characteristic of fully fed "normo-aging" mice to the gene expression profile of slow aging, long term CR mice. In both long and short-term CR mice, changes were observed in gene expression of immune system genes, genes enhancing genetic stability and apoptosis, genes of the enteric nervous system and liver specific genes.

[0055] The methods of the present invention include the identification of interventions that mimic the effects of calorie restriction. Particularly contemplated by the invention are methods of identifying interventions that have an effect on life span, aging, and/or the development of age related diseases and cancer.

[0056] In certain embodiments, such methods comprise obtaining cells, exposing them to an intervention, and observing whether the intervention affects the gene expression profile, levels of RNA, protein, or protein activity related to one or more biomarkers of aging. Preferably, such changes in gene expression, RNA, protein, or protein activity levels would occur within four weeks of the intervention. More preferably, such changes would occur within two weeks of the intervention, and most preferably, such changes occur within two days of the intervention. Such methods permit the identification of pharmacological or other means of achieving a metabolic state similar to the profile observed with long and short-term CR.

[0057] The methods of the present invention include the use of in vitro assays (including gene chip assays) as well as animal assays. Preferably, however, the methods are carried out in live mammals. For example, transgenic mice having enhanced chaperone expression may be used to measure an intervention's ability to reduce cancer, apoptosis, and/or life span. Alternatively, the present methods may be used to identify interventions that mimic calorie restriction simply by measuring the intervention's ability to alter gene expression for a

particular gene or set of genes in live mammals. Such methods allow identification of effective interventions in a short period of time. Interventions identified by the methods of the present invention may be pharmacological, surgical or otherwise. Combinatorial chemistry may also be used in order to screen a large number of pharmacological compounds. In general, the interventions identified by the present invention should be effective in the treatment of cancer, diabetes, age related diseases and/or the extension of life span.

[0058] While the described embodiment represents the preferred embodiment of the present invention, it is to be understood that modifications will occur to those skilled in the art without departing from the spirit of the invention. The scope of the invention is therefore to be determined solely by the appended claims.

EXAMPLES

Example 1

Long Term Calorie Restricted (LTCR) Animals and Treatments for Chaperone Studies

[0059] Female, 28 month old mice of the long lived F, hybrid strain C3Bl0RF$_1$ have been described previously. Mice were weaned at 28 d, housed individually and subjected to one of two diets. The control diet consisted of casein (high protein), 207.0 g/kg, DL-methionine, 4.0 g/kg, dextrose monohydrate, 301.8 g/kg, corn starch, 290.0 g/kg, cellulose, 702. g/kg, brewer's yeast, 8.0 g/kg, Harlan Teklad Vitamin Mix #40060, 10.0 g/kg, Harlan Teklad AIN-76 Mineral Mix #170915, 35.0 g/kg, calcium carbonate ($CaCO_3$), 3.0 g/kg, magnesium oxide (Mg0), 1.0 g/kg, sodium fluoride (NaF), 2.3 mg/kg, sodium molybdate (Na2MoO$\cdot$2H_2O), 0.5 mg/kg. The 50% restricted diet consisted of casein (high protein), 362.0 g/kg, DL-methionine, 7.0ˉ g/kg, dextrose monohydrate, 172.03 g/kg, corn starch, 153.1 g/kg, cellulose, 83.6 g/kg, brewer's yeast, 14.0 g/kg, Harlan Teklad Vitamin Mix #40060, 17.5 g/kg, harlan Teklad AIN-76 Mineral Mix #170915, 61.25 g/kg, calcium carbonate ($CaCO_3$), 5.25 g/kg, magnesium oxide (MgO), 1.75 g/kg, sodium fluoride (NaF), 3.0 mg/kg, sodium molybdate (Na2MoO$\cdot$2H_2O), 0.9 mg/kg. From weaning, control mice were fed 4.8 g of the control diet on Monday through Thursday. On Friday they were fed 13.8 g of control diet. This feeding regimen provided 450 kJ/wk. From weaning, the 50% calorie restricted (CR) mice were fed 4.6 g of the restricted diet on Monday and Wednesday, and 6.9 g on Friday. This regimen provided 225 kJ/wk. Each dietary group received approximately equal amounts of protein, corn oil, minerals and vitamins per gram body weight. The amount of carbohydrates consumed varied between groups. Beginning 30 d before these studies, the control mice were

fed 4.1 g (54.44 kJ) control diet daily at 0900 h. The 50% restricted mice were fed 2.3 g of restricted diet (32 kJ) daily at 0900 h. During this 30 d period, the control and restricted mice received approximately 15% and 50% less dietary energy than normally thought to be required for a typical mouse {Subcommittee on Laboratory Animal Nutrition & Committee on Animal Nutrition 1978 ID: 5480} All food was routinely consumed within 30 min.

[0060] Retired male Swiss-Webster breeder mice were purchased from Jackson Laboratories. Beginning 30 days before the studies, the mice were fed Monday and Wednesday 11 g and Friday 16.6 g of the control diet daily at 0900 h. In fasting-feeding studies, mice were deprived of food for 48 h, fed 5.5 g of the control diet at 0900 h, and killed 90 min later. The food was consumed within 30 min. Diabetes was induced by three weekly intraperitoneal injections of streptozotocin [10 mg/100 g body weight (b.w.)] in 50 mM sodium citrate, pH 4.5. Mice were diabetic one week after the last injection. Only mice with blood glucose level higher than 3 mg/ml were used. Mice injected with equivalent volumes of sodium citrate served as controls for the STZ-diabetic mice. Adrenalectomized and sham operated mice were purchased from Jackson Laboratories. Dibutyryl cAMP (Sigma; 18 mg.100 g b.w.), and theophylline (Sigma; 3 mg/100 g b.w), glucagon (Sigma; 300 μg/100 g, b.w.), dexamethasone (Sigma; 125 μg/100 g b.w), cycloheximide (Sigma; 4 mg.100 g b.w.); and puromycin (Sigma; 10 mg.100 g b.w.), were administered intraperitonealy to mice as specified in the figure legends. Mice received two doses of each drug or drug combination. The first injection was administered 30 min before feeding, and the second injection was administered 30 min after feeding. Mice were killed 1.5 h after the start of feeding. Drug injected mice consumed similar amounts of food as control animals during the feeding period. All animal use protocols were approved by the institutional animal use committee of the University of California, Riverside.

Example 2

RNA Isolation and Quantification for Chaperone Studies

[0061] Mice were killed and the livers, kidneys, and muscle were removed. Muscle from the hind legs and back was removed and pooled for each animal. Tissues were flash frozen in liquid nitrogen. Approximately 0.2 g of frozen tissue was homogenized for 40 s in 4 ml of TRI Reagent (Molecular Research Center, Cincinnati, OH) using a Tekmar Tissuemizer (Tekmar, Cincinnati, OH) at a setting of 55. RNA was isolated as described by the TRI Reagent supplier. RNA was resuspended in FORMAzol (Molecular Research Center) and Northern and dot blots were performed using 20 and 10 μg of RNA respectively. The RNA

was analyzed using Northern blots to verify its integrity. Dot blots were used to quantify mRNA levels (24; 27). Specific mRNA levels were normalized to the level of total RNA and/or mRNA present in each sample using hybridization with radiolabeled complementary DNA to 18S rRNA and/or transcription factor S-II, as indicated in the figure legends (12; 27). The murine ERp72 2.5 kb cDNA was excised with *Bam*HI from pcD72-1 (19). The 1235 bp murine GRP75 coding fragment was excised with *Hind*III from pG7z PBP1.8 (6). A 1.5 kb coding fragment of GRP78 cDNA was produced by digestion of p3C5 with *Eco*RI and *Pst*I (15). A 1.4 kb hamster GRP94 coding fragment was produced by *Eco*RI and *Sa*/K digestion of p4A3 (15). A 664 by coding fragment of rat calreticulin (nucleotides 148 to 812) was produced by PCR from GT10.U1 (23). The entire 2.4 kb cDNA of murine PDI was excised from pGEM59.4 with *Sac*I and *Bam*HI (19). A 1 kb coding fragment of hamster GRP170 cDNA was excised with *Eco*RI and *Xho*I from pCRtmII (16). The 1.9 kb cDNA of murine ERp57 was excised with *Hind*III arid *Sst*I from pERp61 (18). The 1 kb cDNA of murine HSC70 was excised with *Pst*I from phscl.5 (9). The 1.3 kb PEPCK coding fragment was produced by SphI followed by *Sal*I digestions of pGEM5ZEP (a gift from Dr. Garner D.K. Vanderbilt University School of Medicine, Nashville, TN). The fragments were isolated by agarose gel electrophoresis and radioactively labeled using a ^{T7}QuickPrime Kit (Pharmacia) according to the manufacturer's instructions.

Example 3

RNase Protection Assays for Chaperone Studies

[0062] A 223 base pair (bp) DNA fragment made up of 110 bases of intron 3 and all 113 bases of exon 4 of the mouse GRP78 gene was synthesized by PCR using genomic DNA as template and inserted into pT7/T3 (Ambion, Austin, Texas). Two probes of the junction region of intron 7 and exon 7 of the GRP78 gene were produced by PCR using mouse genomic DNA as template. A 257-base fragment including all of exon 7 and the first 113 bases of intron 7 was produced. A 200-base fragment including all of exon 7 and the first 56 bases of intron 7 also was produced. The T7 RNA polymerase promoter was ligated to these PCR fragments using a Lig'nScribe kit as described by the supplier (Ambion). These constructs were used as template for the synthesis of [^{32}P] labeled antisense RNA probes using a MAXIScript kit as described by the supplier (Ambion). RNase protection assays were performed using an RPA II kit as described by the supplier (Ambion). Hybridization of the 257 base RNA probe with GRP78 pre-mRNA protected all 257-bases corresponding to exon 7 and the first 113 bases of intron 7. Hybridization of the 200-base RNA probe to pre-

mRNA protected 200 bases corresponding to all of exon 7 and the first 56 bases of intron 7. Hybridization of either probe to GRP78 mRNA protects the 143-bases complementary to exon 7. A 185- and a 277-bp cDNA fragment of S-II cDNA was synthesized and subcloned into pT7/T3 (12). [^{32}P]-labeled RNA probes for the sense and antisense transcripts were synthesized in vitro and RNase protection assays performed. Hybridization with S-II mRNA protected the entire 185- or 277-base region of the probes. Protection of only the sense strand probes was detected. Quantitation of the hybridized fragments was determined with ImageQuaNT (Molecular Dynamics, Sunnyvale, CA).

Example 4

Plasma Glucose and Insulin for Chaperone Studies

[0063] Plasma glucose, insulin, and glucagon concentrations were determined using Glucose [HK] 10 (Sigma, St. Louis, MO), Rat Insulin RIA and Glucagon RIA kits (Linco Research, St. Charles, MO), as described by the suppliers.

Example 5

Statistical Analysis for Chaperone Studies

[0064] The data shown in Figure 1 are expressed as means ± SD for 5 mice at each time point. The effects of food deprivation and subsequent feeding on mice of each dietary group were analyzed using a one way ANOVA followed by Fisher's test. The analysis determined whether individual time point means differed from time 0 means within each dietary group. It also determined the differences between the means of the control and CR groups at each time point. Differences of $P < 0.05$ were considered significant. Values are expressed as means ± SD. Significance was determined with either Student's unpaired t-test ($P<0.95$) or a one way ANOVA followed by Fisher's or Tukey's tests ($P<0.01$). All statistical analyses were performed with Minitab Statistical Software (Minitab, State College, PA).

Example 6

Chronic and Acute Effects of Calorie Consumption on Hepatic Chaperone mRNA

[0065] Feeding of the fasted mice rapidly induced the abundance of GRP78 and ERp72 mRNA (Figures 1A and 1B). A large increase in chaperone mRNA was detected by 1.5 h after feeding, the first time point studied. The 24 h fasting levels (0 time) of GRP78 and ERp72 mRNA were lower in the CR mice. The response to feeding was kinetically different in control and CR mice. Thus, the amount of food consumed affects the kinetics of the

response. The integrated level of GRP78 and ERp72 mRNA over the entire 24-hour period was also less in the CR than in control mice. Similar results were obtained when the effects of feeding on HSC70, ERp57, and calreticulin mRNA were determined (data not shown). Thus, this represents a common response of chaperone gene expression to feeding.

Example 7

Fasting Feeding Induced Multiple Chaperone mRNAs in Multiple Tissues

[0066] Mice were fasted for 48 hours and refed for 1.5 hours. Hepatic GRP78 mRNA was induced approximately 3-fold after this time (Figure 2A). The mRNA for the other ER chaperones investigated, ERp57, ERp72, GRP94, GRP170, PDI, and calreticulin, and for the most abundant cytoplasmic chaperone, HSC70, also were induced by feeding (Figure 2A). HSC70 was induced by nearly 3-fold. No changes in the mitochondrial chaperone GRP75 was detected in this study. By examining chaperone levels in other tissues of fasted and fed mice, we found that the feeding-related chaperone induction extends to at least kidney and muscle (Figure 2B). GRP78 mRNA induction is shown in the figure (Figure 2B). HSC70 mRNA was also induced in these tissues (data not shown). In studies not shown, we have found that a similar induction of hepatic chaperone mRNAs occurs in rat. Thus, the response is shared by other species.

Example 8

CR Reduces the Abundance of the GRP78 Primary Transcript

[0067] RNase protection studies were used to investigate the responsiveness of the GRP78 mRNA and primary transcript to chronic differences in dietary calorie consumption. A probe was utilized for these studies designed so that the GRP78 primary transcript protected a 223 base RNA fragment representing the third intron-fourth exon boundary of the transcript (Figure 3A lane 1, upper band). The mRNA protected a 113 base fragment of the probe which represents the fourth exon of the gene (Figure 3A, lane 1, lower band). Much less of the 223 and 113 base GRP78 precursor and mRNA probes were protected by RNA from CR mice (Figure 3A, lanes 4-9). A probe for 185 bases of S-II mRNA was included in each sample as an internal control (Figure 3A, lane 3). S-II mRNA is unresponsive to CR or fasting feeding (25). The unlabeled bands in Figure 3 represent RNase resistant artifacts of the S-II probe (Figure 3A, lane 2).

[0068] When the amount of protected probe was quantified and normalized to the signal obtained from the S-II probe, it became clear that the abundance of the chaperone precursor

and mRNA were decreased to the same extent in the CR mice (Figure 3B). The same conclusion was reached using a probe for the boundary regions of intron 7 and exon 7. Consequently, CR decreases either the rate of GRP78 gene transcription or the stability of the GRP78 primary transcript. The data are not consistent with blocked or paused GRP78 gene transcription or changes in the stability of the mRNA in CR mice.

Example 9

Fasting feeding induction of the GRP78 primary transcript

[0069] RNase protection studies also were used to investigate the fasting feeding response. RNA isolated 1.5 h after feeding protected much more of a 257 base fragment representing the exon 7-intron 7 boundary of the primary transcript than RNA isolated from fasted mice (compare Fig. 4A, lanes 10-12 to lanes 7 9). Similar results were obtained with a probe in which 200 bases representing the exon 7-intron 7 boundary were protected (compare Fig. 4A, lanes 16-18 to lanes 13-15). In each case, RNA from refed mice also protected more of the 143 base fragment representing the exon 7 region of the mRNA (Fig. 4A). A probe for 277 by of the S-II mRNA was present in each assay for use as an internal control.

[0070] Quantification of these data, and normalization of the S-II internal control demonstrated that the mRNA and the precursor RNA were induced by feeding to essentially the same extent (Fig. 4B and 4C). Similar results were obtained using the probe described earlier for the third intron fourth exon boundary of the gene (data not shown). Without being bound to a specific mechanism, these data suggest the same molecular step is responsible for regulating the genetic responsiveness of chaperones to both acute and chronic changes in calorie consumption. This mechanism appears to involve changes in either the transcription or the stability of the primary transcript.

Example 10

Inhibitors of protein synthesis

[0071] To investigate the physiological basis for the fasting feeding response, studies were performed using inhibitors of protein synthesis. Fasted mice were treated with a dose of cycloheximide or puromycin sufficient to inhibit greater than 95% of protein synthesis in the liver. Treatment with cycloheximide strongly induced GRP78 mRNA in fasted mice (Fig. 5A). GRP78 mRNA also was strongly induced in cycloheximide-treated, refed mice. Puromycin treatment modestly induced GRP78 mRNA in fasted mice (Fig. 5A). Feeding of puromycin treated mice fully induced the mRNA. Thus, induction by feeding does not

appear to require de novo protein synthesis. Further, these results suggest that the lower chaperone mRNA levels in fasted mice may involve the action of a rapidly turning over factor.

[0072] The effects of the protein synthesis inhibitors on PEPCK mRNA also was determined as a positive control. The effects of fasting feeding and cycloheximide treatment on this mRNA are well known. Fasting induced, and feeding repressed PEPCK mRNA, as expected (Fig. 5B). Also, as expected from published data, cycloheximide increased PEPCK mRNA in both fasted and refed mice through its effects on PEPCK mRNA stability. The effects of the inhibitors on PEPCK mRNA levels indicate the inhibitors were efficacious in these studies.

Example 11

Pancreatic hormones and glucose

[0073] The physiological hallmarks of the fasting feeding transition are increased circulating insulin and decreased circulating glucagon. In the studies shown in Fig. 6, fasted and refed sham injected mice had serum glucose concentrations of 84.4 ± 5.1 and 121.1 ± 8.0 mg/dl, serum insulin concentrations of 0.491 ± 0.203 and 1.3 ± 0.256 pmol/ml, and serum glucagon concentrations of 143 ± 22.4 and 81.4 ± 13.2 pg/ml, respectively.

[0074] To investigate whether these hormones are involved in the postprandial induction of GRP78 mRNA, the effects of cAMP, glucagon, and STZ-induced diabetes on the response were examined. Administration of either dibutyryl cAMP or glucagon reduced the response of GRP78 mRNA to feeding (Fig. 6A). Vehicle alone had no effect. Likewise, STZ induced diabetes resulted in a blunted response to feeding although it did not modify the fasting level of GRP78 mRNA. When STZ-induced diabetes was combined with cAMP administration, the postprandial induction of GRP78 mRNA was obliterated. The mRNA remained at fasting levels. Without being bound to any particular mechanism, these results suggest that glucagon, acting to increase intracellular cAMP levels, suppresses chaperone gene transcription, or possibly GRP78 pre-RNA stability. Further, they suggest that insulin is required for full responsiveness of the chaperone genes to decreased intracellular cAMP.

Example 12

Luminal filling

[0075] Luminal filling can lead to the release of some gastrointestinal polypeptides. For this reason, we investigated the role of luminal stimuli on the chaperone mRNA response.

Fasted mice were refed a nonnutritive paste of cellulose (a normal component of their regular diet) and mineral oil. The mice initially consumed the mixture enthusiastically. Stomach filling was confirmed for each mouse by postmortem examination. Cellulose-mineral oil consumption produced a minor but significant increase in GRP78 mRNA (Fig. 6B), without producing a change in plasma glucose, insulin, or glucagon concentrations.

Example 13

Adrenal hormones

[0076] To investigate the role of adrenal hormones in the postprandial induction of GRP78 mRNA, we examined the effects of feeding in adrenalectomized mice (Fig. 7). Neither adrenalectomy nor sham surgery had any effect on the fasting levels of GRP78 mRNA. However, adrenalectomy increased the magnitude of the postprandial induction of the mRNA by approximately 2-fold over that found in refed, sham operated mice. The feeding response of GRP94, ERp72, and GRP170 were also enhanced in the adrenalectomized mice (data not shown). Thus, the increase is a generalized ER chaperone response. Administration of dexamethasone to adrenalectomized mice increased the basal level of GRP78 mRNA during starvation, although not significantly (Fig. 7). However, dexamethasone administration had no effect on the feeding induction of the gene, suggesting its absence from adrenalectomized mice is not responsible for the enhancement of the feeding response.

Example 14

Preparation of test groups for short-term CR studies

[0077] Three groups of 30 month old mice were utilized for these studies. Male $B6C3F_1$ mice were maintained as described (Dhahbi et al. (1998) J. Gerontol 53A: B180). Mice were weaned at 28 days and housed individually. The composition of the defined diets used have been described. They are formulated so that only the amount of carbohydrate consumed varied between the CR and control mice. A group of control mice was fed a purified, semi-defined diet from 6 weeks of age. Control mice consumed approximately 105 kcal per week from weaning. This is approximately 10% less than the amount of food thought to support optimal growth, fertility and fecundity in mice {Subcommittee on Laboratory Animal Nutrition & Committee on Animal Nutrition 1978 ID: 5480}. Subjectively, these mice appeared neither fat or lean. A group of calorically restricted mice (CR mice) were fed a diet reduced in dietary carbohydrate such that the mice consumed approximately 40% fewer calories than control mice. The long term CR mice consumed approximately 55 kcal per

week from wearing. The short-term CR mice were fed 105 kcal until the age of 29 months. They were then fed 80 kcal of control diet for 2 weeks, followed by 55 kcal of CR diet for two weeks. The mice were fed daily at 0900 hours. They had free access to water. For the studies, mice were fed a normal allotment of food Monday morning, and all the food was eaten within 45 minutes. They were fasted for 24 hours, and killed on Tuesday morning. At the time of use, the long term CR, short-term CR and control mice weighed 22.8 ± 1.4, 25.2 ± 0.3 and 37.2 t 2.4 g, respectively. The mice were approximately 30 months old when killed.

[0078] Mice were killed by cervical dislocation and the liver rapidly removed and flash frozen in liquid nitrogen. Approximately 0.2 g of frozen liver was homogenized for 40 s in 4 ml of TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH) using a Tekmar Tissuemizer (Tekmar Co., Cincinnati, OH) at a setting of 55. RNA was isolated as described by the supplier.

[0079] GeneChip oligonucleotide based high-density array RNA expression assays were performed according to the standard Affymetrix protocol. The biotinylated, fragmented cRNA was hybridized to the Mu11KsubA and Mu11KsubB GeneChip arrays (Affymetrix, Santa Clara, CA), which contain targets for more than 11,000 known mouse genes and ESTs. The arrays were washed, stained and scanned. Scanned image analysis and data quantification were performed using the Affymetrix GeneChip analysis suite v3.2 at default parameter settings. Resultant data were normalized by global scaling.

[0080] **Data analysis.** Data sets were normalized further using GeneSpring 3.0 (Silicon Genetics, San Carlos, CA). Negative expression levels were forced to zero, and the expression data for each animal divided by the median of all experimental values for that chip above an expression level of 10. This step reduced chip-to-chip signal variation. Fold change in expression was calculated by dividing the mean of the expression levels in the CR groups by the mean of the expression levels in the control group.

[0081] **Statistical analysis**. To test for significance of the effect of diet on gene expression, one way ANOVA was followed by Fisher's test ($P < 0.05$). Genes were placed in expression pattern groups (Table 2) for which they passed both tests. All statistical analyses were performed using Minitab Statistical Software.

Example 15

Gene Expression in long and short-term CR mice

[0082] The global patterns of hepatic gene expression in the three groups of mice as displayed by GeneSpring 3.0, are shown in Fig. 8. The 11,000 genes assayed in the study are

grouped according to both structure and function by the GeneSpring gene clustering algorithm across the horizontal axes of the figure. While this representation of the data cannot be subjected to statistical tests, subjective examination of this color coded representation of the data obtained immediately suggests that striking similarities exist in the gene expression profile of long and short-term CR mice. Likewise, examination of the figure suggests that both CR expression profiles are very different than the profile of control mice. An average linkage hierarchical clustering dendrogram calculated from the data by the GeneSpring clustering algorithm is shown to the left of the expression profiles. The dendrogram shows that the algorithm clustered the short- and long term CR groups together, separated from the control group. This analysis agrees with our subjective interpretation of the expression profile.

[0083] Another aspect of this representation of the data was of interest. Significantly larger areas of blue were found in the expression profile of the control mice. These areas represent genes for which expression was not detectable. In both groups of CR mice, many of these regions were red, indicating higher levels of expression. Thus, a major effect of CR was the activation of specific gene expression.

[0084] To quantify the similarities in gene expression among groups of mice, a global expression correlation coefficient was calculated for each possible pair of mice. Table 1 shows the nine by nine matrix of these pairwise comparisons. The values are a measure of the similarities in gene expression between pairs of mice. Because the mice were genetically identical, the intra group values provide a measure of the maximum correlations attainable. The inter group correlations of the short- and long term CR mice were similar to their intra group correlations, indicating that gene expression in all CR mice was similar. In contrast, the control mice have little correlation with the mice in either CR group. This analysis suggests that short- and long-term CR had highly similar effects on overall patterns of specific gene expression.

[0085] Table 1. Pairwise comparisons of the global gene expression correlation coefficient calculated for each possible pair of mice.

	CR	CONTROL	SWITCHED
CR	1.00* 0.25 0.32	0.01 0.04 -0.04	0.16 0.17 0.18
	1.0 0.27	-0.03 0.03 -0.01	0.13 0.12 0.18
	1.00	0.02 0.02 -0.02	0.18 0.14 0.21

CONTROL		1.00 0.29 0.42 1.00 0.28 1.00	0.0 0.03 0.07 0.07 0.10 0.01 -0.02 0.02 0.05
SWITCHED			1.00 0.24 0.18 1.0 0.16 1.00

Example 16

Long- and short-term CR induced expression of the same genes

[0086] The pseudogene function of GeneSpring 3.0, and statistical analysis of the data were utilized to sort the genes into one of seven possible categories of relative gene expression. These groups were: expression not different among groups; expression high in long term CR, low in control, and high in short-term CR (termed, *high-low-high*) (Appendix A); expression low in long term CR, high in control, and low in short-term CR (*low- high- low*) (Appendix B); expression low in long term CR and control, but high in short-term CR (*low-low-high*) (Appendix C); expression high in long term CR and control, and low in short-term CR (*high-high-low*) (Appendix D); expression high in long term CR, and low in control and short-term CR (*high-low-low*) (Appendix E); and expression low in long term CR and high in control and short-term CR (*low-high-high*) (Appendix F). The vast majority of the genes were not different among groups, and will not be discussed further.

[0087] Table 2 shows the number of genes and expressed sequence tags (ESTs) in each of the other groups. Ninety percent of these genes and ESTs were in the high-low-high and low-high-low groups. In these groups, the short- and long-term CR expression patterns are most similar. The other 4 groups accounted for only 10% of the remaining genes and ESTs. These data indicate that short- and long-term CR produced remarkably similar effects on the expression of more than 11,000 hepatic genes and ESTs. A complete listing of the expression data for the genes and ESTs in each group is available (http://www.biochentistry.ucr.edu/faculty/ spindler.html/GeneChipData) (This URL will be activated upon allowance of this application).

[0088] By far the most common response to short- and long-term CR was the high-low-high expression pattern. It accounted for nearly 86% of the genes and ESTs in the groups. Thus, the most common effect of short- and long-term CR was the activation of gene expression. To determine whether short- and long-term CR induced expression to the same

degree in the high-low-high group, we tabulated the number of known genes for which expression was statistically the same in the two groups. In high-low-high, 303 of 340 known genes (89%) were expressed at the same level in the short- and long term CR groups. For 26 of these genes (8%), expression in the long term CR mice was statistically greater. For 11 genes (3%), expression was greater in the short-term CR group. Thus, short- and long-term CR induced the expression of the vast majority of these genes to the same levels.

[0089] Of the genes in the high-low-high group, 146 of 340 genes were activated from undetectable levels in the control mice to much higher, but very similar levels in both CR groups. Expression of these genes averaged 1.25 ± 0.25 and,1.23 ± 0.23, in the short- and long-term CR groups, respectively. These observations reinforce the idea that short- and long-term CR have highly homologous effects on the expression of genes.

[0090] To further understand the genomic effects of CR, we identified the genes in the high-low-high group described above.

TABLE 2. GENES WHICH DIFFER FROM CONTROL IN RESPONSE TO CR

LT CR*	CONTROL	ST CR**	GENES	EST's	PER CENT
High	Low	High	340	860	85.7
Low	High	Low	23	37	4.3
High	High	Low	4	9	0.9
Low	Low	High	13	19	2.3
High	Low	Low	26	55	5.8
Low	High	High	9	6	1.1

* Long term CR

** Short-term CR

Example 17

Immune system activation: The immune theory of aging

[0091] Many of the genes which were induced by CR in the long and short-term CR group were genes involved with immune system activation. Without being limited to any specific mechanism, this result provides support for the theory that the immune system plays a central role in the rate and many of the pathologies of aging. Slightly more than 130 T-cell receptor, IgG, IgA, IgD, IgK, and IgM, genes were present in the high-low-high group. The average

fold relative expression of these mRNAs in the long and short-term CR. groups was 1.24 ± 0.86 and 1.23 ± 0.25, verses 0.16 ± 0.16 in the control group. Thus, CR increased immunoglobulin and T-cell receptor expression more than 10 fold. It is highly unlikely that this increase was due to an increase in the amount of blood in the CR livers. The level of globin mRNA found in these mRNA samples was actually reduced by about 20% in the long and short-term CR groups. No statistically significant difference was found in the globin mRNA concentration in the blood of these animals.

[0092] Other changes in gene expression indicate that CR activates the immune system (Table 3). As can be seen in the table, both long and short-term CR induced the expression of hemopoietic and lymphopoetic cytokines, hormones, signal transduction proteins, protein kinase modulators of the cell cycle and signal transduction, cell surface receptors, and transcription factors. Not shown are a group of 20 immune cell specific genes known to be involved in endocytosis, cell adhesion, phagocytosis, potassium channels, lymphocyte activation, VDJ recombination, and immune cell activation which were strongly and significantly induced by CR (3- to 40-fold; $P \geq 0.037$). Together, these data evidence that CR enhances the activity of the immune system.

Table 3. Immune system genes activated by short- and long-term CR

LTCR*	STCR*	*P*	GENE
			Hormones/Cytokines/Chemokines
4	4	0.003	Antigen, B cell receptor; L43567
53	55	<0.001	Calcium/calmodulin-dependent protein kinase IV (*Camk4*); multifunctional serine-threonine protein kinase; T cells; X58995
>100	>100	<0.001	Chemokine (C-C) receptor 1 (*Cmkbr1*); growth inhibitory effects; liver and spleen; U28404
13	17	<0.001	Chemokine (C-C) receptor 5 (*Cmkbr5*); induces mobilization of intercellular calcium; beta-chemokine; leucocyte chemoattractant; liver, thymus, spleen, elsewhere, ET62976
>100	>100	0.003	Chemokine (C-X-C) receptor 4 (*Cmkbr4*); integral membrane G-protein-coupled receptor; chemotaxis and calcium flux; directs monocytes and lymphocytes to their target tissues; thymus, T cells, and monocytes; ET62920
19	21	0.002	Colony stimulating factor 1 (macrophage) (*Csf1*); receptor; liver; X06368
10	8	0.016	Complement receptor 2 (*Cr2*); Late pre-B cells; M35684
3	2	0.015	Interferon beta type 1; growth factor; T helper cell differentiation factor; antiviral; modulates immune response to foreign and self-antigens; immune system cells, others; V00755
11	10	<0.001	Interferon-related developmental regulator (*Ifrd1*); T cells; V00756

LTCR*	STCR*	*P*	GENE
9	6	0.044	Interleukin 2 (*Il2*); stimulates proliferation of activated T lymphocytes; M16762
>100	>100	0.015	Interleukin 2 receptor (*Il2r*); T cells; M26271
2	2	0.014	Interleukin 6 (*Il6*); promotes B cell maturation to Ig-secreting cells; activation of T cells; some helper T cells and macrophages; X54542
5	6	0.004	Interleukin 7 (*Il7*); growth factor; B cell progenitors; X07962
4	3	0.046	Killer cell lectin-like receptor, subfamily A, member 3 (*Klra3*); Ly-49C; involved in graft rejection; subpopulation of natural killer cell; U49866
>100	>100	0.034	Killer cell lectin-like receptor, subfamily A, member 6 (*Klra6*); Ly-49F; NK cell surface antigen; determinant of IL-2-activated NK cell specificity; inhibitory receptor for interaction with MHC class I proteins; NK cells; U10092
13	11	<0.001	Lymphocyte antigen 84 (*Ly84*); signal transduction protein 2; T cells; D13695
5	6	0.007	Mast cell protease 7 (*Mcpt7*); released when mast cells are activated; mast cells; ET61471
3	2	0.037	Myc box dependent interacting protein 1 (*Bin1*); endocytosis and signal transduction; recycling synaptic vesicle components; macrophages, neurons, endocrine cells; U86405
>100	>100	<0.001	Paired-Ig-like receptor A1 (*Pira1*); activates B lymphocytes, dendritic and myeloid-linage cells; ET62839
5	4	0.027	Paired-Ig-like receptor A6 (*Pira6*); appears to activate immunoglobulin-related receptor; B lymphocytes, myeloid lineage cells; ET62844
3	4	0.038	Preprosomatostatin (*Smst*); regulates T cell IFN-gamma production; macrophages, nervous system; X51468
>100	>100	<0.001	Protein tyrosine phosphatase, receptor type E (*Ptpre*); transmembranal, receptor-like form and a cytoplasmic, non-receptor form; hematopoietic tissues; ET61424
23	41	0.010	Proviral integration site (*Pim2*); serine/threonine kinase 2; cell proliferation; mitogen stimulated; long-term potentiation in hippocampus; immune and epithelial cells, CNS; L41495
			Receptors/Signal Transduction Proteins
11	8	0.001	Small inducible cytokine subfamily, member 2 (*Scyb2*); small inducible cytokine; macrophages; X53798
8	8	0.002	Son of sevenless 1, homologue 1 (Drosophila) (*Sos1*); Ras-specific exchange factor; T cells; Z11574
>100	>100	<0.001	Son of sevenless 2, homologue 2 (Drosophila) (*Sos2*); Ras-specific exchange factor; T cells; Z11664
>100	>100	0.002	Spleen protein kinase (*Syk*); signal transduction; lymphopoietic and haematopoietic cells, platelets, macrophages and neutrophils; ET61263
>100	>100	0.048	*Tbcl*; domains homologous to *tre*-2 oncogene and yeast mitosis regulators BUB2 and cdc16; nuclear localization; B lymphocytes; dendritic cells, myeloid-linage cells; U33005
2	2	0.044	Thrombin receptor; transmembrane G-protein-coupled receptor; activated by serine protease cleavage; mitogen and

LTCR*	STCR*	*P*	GENE
			apoptosis inducer following vessel injury; platelets, monocytes, endothelial cells, neuronal and glial cells; U36757
>100	>100	0.002	Weel homologue (S. pombe) (*Weel*); inhibits entry into mitosis by phosphorylation of the Cdc2 kinase; lymphocytes; D30743
			Transcription Factors
38	35	<0.001	Abelson marine leukemia oncogene (*Abl*); nonreceptor tyrosine kinase; role in cell cycle progression, cell proliferation and differentiation; liver, B cells, others; X07540
>100	>100	0.047	Homeo box A4 (*Hoxa4*); transcription factor; embryonic spinal core and adult testis; X13538
4	7	0.026	Homeo box B4 (*Hoxb4*); transcription factor; embryonic development; haematopoiesis; NK cells; M36654
6	10	0.029	Homeo box B7 (*Hoxb7*); transcription factor; embryonic development; haematopoiesis; developing embryo; blood, bone marrow, natural killer cells; X06762
8	9	<0.001	Homeo box C6 (*Hoxc6*); transcription factor; embryogenesis haematopoiesis; liver and many other tissues; X16510
40	36	0.001	Homeo box D1 (Hoxdl); transcription factor; neurogenesis; developing CNS and forelimb bud; X60034
>100	>100	<0.001	Nuclear factor of activated T cells, cytoplasmic 2 (Nfatc2); T cell transcription factor isoform B; T cells; U36575
5	5	0.001	SRY-box containing gene 4 (Sox; Sox gene family transcription factor; thymus, bone marrow, gonads; ET62444
2	2	0.012	Zinc finger protein 79 (Zfp79); Kruppel type zinc finger putative transcriptional repressor; associates with RB in vitro; hematopoietic cells, perhaps others; U29513
			Primary Response Genes
>100	>100	0.005	Fos-like antigen-1 (Fosll); spleenocytes; U34245
.100	>100	<0.001	<0.001Immunity associated protein, 38 kDa (Imap38); spleenocytes; Y08026
>100	>100	<0.001	Immunoresponsive gene 1(Irgl); activated by bacterial LPS treatment; macrophages; L38281
>100	>100	<0.001	Prostaglandin-endoperoxide synthase (Ptgs2); putative mediator of inflammation; induced by growth factors and cytokines; monocytes and fibroblasts; M88242
388	353	0.001	T-cell acute lymphocytic leukemia 2 (Ta12); putative basic helix-loop-helix transcription factor activated in T-cell acute lymphoblastic leukemia; T cells; M81077
>100	>100	<0.001	Tumor necrosis factor induced protein 3 (Tnfip3); putative helix-loop-helix transcription factor activated in T-cell acute lymphoblastic leukemia; lymphocytes; U19463
			Cell Adhesion / Membrane Components
>100	>100	0.002	ADP-ribosyltransferase 2a (Art2a); homologue of the rat T cell differentiation marker RT6; cell-cell signaling; cytotoxic T lymphocytes; X52991
9	9	0.013	Cadherin 9 (Cdh9); calcium-binding membrane glycoprotein; cell adhesion molecule; thymocytes; U69136
6	5	0.015	CD22 antigen (Cd22); mediates B cell interactions with

LTCR*	STCR*	*P*	GENE
			endothelial cells; B cells; L16928
7	7	0.002	CD53 antigen (Cd53); pan-leukocyte antigen; cell membrane glycoprotein; thymocytes; X97227
40	36	<0.001	Erythrocyte protein band 7.2 (Epb7.2); involved in Na+/K+ permeability of cells; spleen, lung, testis; X91043
8	8	0.006	Integrin alpha 4 (Itga4); cell adhesion; lymphocytes; X53176
>100	>100	<0.001	Moose receptor, C type 2 (Mrc2); cell adhesion; antigen presentation; widespread tissue distribution, fetal liver; U56734
			Immune Cell Function
38	44	<0.001	Cytochrome b-245, beta polypeptide (Cybb); gp9lphox; flavocytochrome mediating electron transfer from NADPH to molecular oxygen in the respiratory burst oxidase; phagocytes; U43384
8	8	<0.001	Cytotoxic T lymphocyte-associated protein 2 beta (Ctla26); homologue of cysteine protease proregion; T cells; X15592
>100	>100	<0.001	GranzymeG (Gzmg); CTL serine protease 3; may play a role in cytolytic lymphocyte activation; T lymphocytes; X14092
>100	>100	0.007	Helicase, lymphoid specific (Hells); replication, repair, recombination and transcription; T and B cells; U25691
>100	>100	0.001	Mgt cell protease 4 (Mcpt4); secretory granule serine protease; peritoneal and most connective tissue mast cells; M55617
5	6	0.007	Mast cell protease 7 (Mcpt7); released when mast cells are activated; mast cells; ET61471
8	8	0.005	Potassium voltage gated channel, shaker related subfamily, member 2 (Kcna2); T cells, myelinating Schwann cells; M30440
3	3	0.003	Terminal deoxynucleotidyl transferase (Tdt); VDJ assembly; recombination; earliest stage B and T cells; X04123

*Fold of control

[0093] Further support for this view was found in the liver specific genes which were strongly induced in expression by CR (Table 4). Long and short term CR significantly enhanced the expression of the CD44 hyaluronan receptor gene, which has a role in lymphocyte homing and activation. Likewise, CR activated the mRNA abundance of the chemokine receptor 4, which is also involved in stimulating growth of pre B cells; the mannose receptor, C type 2, which is involved in antigen presentation; colony stimulating factor 1, which is a macrophage growth factor; and proteaseome 3, which enhances the generation of class I binding peptides.

LTCR*	STCR*	*P*	GENE
			Cytokines/Growth Factors
12	7	0.003	C-Fos induced growth factor (Figs; secreted growth factor; mitogenic and morphogenic activity; endothelial cells of liver

LTCR*	STCR*	*P*	GENE
			during embryonic development; X99572
2	2	0.002	Fibroblast growth factor 2 (FgfZ); mitogen, differentiation and survival factor, angiogenic factor; stimulates hepatocyte proliferation and migration; hepatocytes, other cells; M30644
>100	>100	0.001	Fibroblast growth factor 3 (Ffg3); liver epithelial cells; Y00848
3	3	0.012	Fibroblast growth factor 7 (Fgf7); liver epithelial cells; ET62118
>100	>100	0.001	Follistatin (Fst); binds and inactivates activin; control of the inflammatory cascade; liver; 229532
>100	>100	0.005	Inhibin beta B (Inhbb); transforming growth factor beta (TGF-beta) superfamily member; liver and elsewhere; X69620
>100	>100	0.001	Inhibin beta E (Inhbe); transforming growth factor beta (TGF-beta) superfamily member; liver and elsewhere; U96386
13	9	0.000	Interferon alpha gene family leukocyte (Infa); inhibition of cell proliferation; ubiquitous; M28587
3	2	0.015	Interferon beta type 1; growth factor; T helper cell differentiation factor; antiviral; modulates immune responses to foreign and self-antigens; ubiquitous; V00755
11	11	0.001	Interferon-beta (Ifnb); inhibitor of inflammation; liver and other cells; J00424
13	13	<0.001	Neurotrophin 3 (NV3); secreted protein; binds high affinity receptor trk C; may be involved in postnatal development; liver parenchyma) cells, cerebellum, thymus, other; X53257
4	5	0.003	Preproendothelin 1 (Ednl); activates p38 MAP kinase and 7NK; portal vein constriction; hepatic stellate cells, liver and arterial smooth muscle cell, others; U07982
10	15	0.003	Transforming growth factor, beta 2 (Tgjh2); cell proliferation; liver stellate cells; X57413
			<u>Cell Surface Receptors</u>
>100	>100	0.020	Bradykinin receptor beta (Bdkrb); G-protein-coupled membrane bound; T-kininogen modulation during acute phase protein synthesis; liver (ubiquitous); ET61 S 59
2	2	0.017	CD44 antigen (Cd44); receptor for hyaluronan; cell surface glycoprotein; hyaluronan clearance from the blood; lymphocyte homing and activation; liver, CNS, other; U57612
>100	>100	<0.001	Chemokine (C-C) receptor 1 (Cmkbrl); mediates growth inhibitory effects of the chemokine; liver and spleen; U28404
12	8	0.013	Chemokine (C-X-C) receptor 4 (Cmkar4); primary receptor stromal cell-derived factor/pre-B growth stimulating factor; seven transmembrane domain receptor; liver and bone marrow; X99581
>100	>100	<0.001	Fibroblast growth factor receptor 2 (Fgfr2); membrane-spatming tyrosine kinase; activated by three members of `the FGF family; liver development; liver parenchyma) cells and others; M86441
4	3	0.001	Leptin receptor (Lepr); transmembrane receptor; liver, lung, muscle, brain, other; ET61693
4	3	0.027	Melanocortin 5 receptor (Mc.ir); G-protein-coupled receptor;

LTCR*	STCR*	*P*	GENE
			stimulates adenylyl cyclase; widely expressed; X7629
3	4	0.029	Pancreatic polypeptide receptor 1 (Ppyrl); neuropeptide Y; peptide YY receptor; G-protein-coupled; liver; U40189
>100	>100	<0.001	Proteaseome 3 (Psme3): Ki antigen; cell proliferation; enhances generation of class I binding peptides; liver, broad tissue distribution; U60330
>100	>100	<0.001	Purinergic receptor P2X, ligand-gated ion channel 1 (P2rx1); mediate Ca(2+) influx; liver, ubiquitous; X84896
64	68	0.001	Ryanodine receptor 2 (Ryr2); endoplasmic reticulum membrane Ca2+ channels; controls cytosolic calcium levels; liver, cardiac muscle, neurons, most excitable cells; X83933
>100	>100	0.003	Transferrin receptor (Trfr); cell surface glycoprotein; cell growth; iron uptake; liver; X57349
			Signal Transduction/Cell Cycle/Cell Growth
38	35	<0.001	Abelson marine leukemia oncogene (Abn; nonreceptor tyrosine kinase; role in cell proliferation and differentiation; liver, B cells; X07540
>100	>100	0.006	Cyclin-dependent kinase inhibitor 1B (P27) (Cdknlb); cell cycle; ubiquitous; U10440
35	40	0.003	Guanine nucleotide binding protein, alpha inhibiting 1 (Gnail); liver, cerebral cortex, others; U38501
>100	>100	0.013	Guanine nucleotide binding protein beta 4 (Gnb4); liver, brain, blood cell; M63658
>100	>100	0.001	Histamine receptor H1(Hrhl); coupled to phosphoinositide turnover-calcium mobilization signaling pathway; regulates IGF-I expression and cell proliferation; regulates thyroxine transport into hepatocytes; liver, brain, spleen (ubiquitous); D50095
>100	>100	0.002	Interferon-activated gene 204 (Ifc204); mediates antimicrobial, immunomodulary and cell growth-regulatory activities of interferons; nucleoli; M31419
4	4	0.004	Kinase interacting with leukemia-associated gene (Kis); cytosolic phosphoprotein; integration of intracellular proliferation and differentiation signaling; ubiquitous; X82320
9	8	0.004	MAD homologue 5 (MadhS); downstream component in the TGF-beta family signaling cascade; liver development angiogenesis; liver; ET62570
>100	>100	0.002	MAP kinase kinase kinase (iVfap3kl); serine-threonine kinase; regulates sequential protein phosphorylation pathways involving mitogen-activated protein kinasss (MA.PKs); ubiquitous; ET61257
>100	>100	0.002	Mitogen activated protein kinase 1 (Mapkl); signal transduction; cell proliferation, differentiation, and apoptosis; liver, ubiquitous; U85608
>100	>100	0.004	NIMA_related expressed kinase (1Vek1); ubiquitous; 54828
3	3	0.041	Neuroblastoma ras oncogene (Nras); key component of growth signaling pathways; liver, wide tissue distribution; X13664
>100	>100	<0.001	Phosphatidylinositol 3-kinase regulatory subunit, polypeptide

LTCR*	STCR*	*P*	GENE
			1 (p85alpha) (Pik3r1); role in cell growth, differentiation, survival, and vesicular transport; liver; ET61628
>100	>100	0.003	Phospholipase C, gamma 1 (Plcgl); produces second messengers of signal transduction pathways related to cell proliferation; ubiquitous; ET63005
>100	>100	<0.001	Proteaseome 3 (Psme3); Ki antigen; cell proliferation; enhances the generation of class I binding peptides by altering the cleavage pattern of the proteosome; liver, neurons, broad tissue distribution; U60330
3	2	0.002	Protein tyrosine phosphatase, non-receptor type 16 (Ptpnl 6); growth factor-induced immediate early gene; dephosphorylates MAP kinase; liver parenchyma) and vascular smooth muscle cells, others; X61940
11	12	0.001	Ras-GTPase-activating protein SH3-domain binding protein 2 (G36p2 pending); essential for Ras signaling; ubiquitous; U65313
2	2	0.001	Rhodopsin kinase (Rhok); small GTPase and serine/threonine protein kinase; regulates actin cytoskeletal reorganization; enhances secretion; ubiquitous except for brain and muscle; U58513
15	14	0.018	Ros 1 proto-oncogene (Rosl); embryonic development; tyrosine kinase catalytic domains; expressed in neoplastic and fetal tissues; neoplastic and fetal tissues; U15443
6	4	0.010	SUMO-1 activating enzyme subunit 1; conjugates SUMO-1 (a small ubiquitin-like protein) to other proteins; modification of I Kappa B alpha blocks NF kappa B-dependent transcriptional activation; ubiquitous; AA162130
>100	>100	<0.001	Wingless related MMTV integration site lOb (WntlOb); developmental regulation of cell growth and differentiation; ET62229
			Nuclear Receptors
19	17	0.016	0.016 Thyroid hormone receptor alpha (Thra); energy balance, thermoregulation, substrate uptake; liver; X07751
10	9	0.003	Glucocorticoid receptor 1 (Grll); energy balance; substrate uptake; liver; X04435
45	42	<0.001	Nuclear receptor subfamily 2, group F member 1 (Nr2f1); COUP-TFI; orphan steroid hormone receptor; transcription factor; liver; X74134
>100	>100	0.010	Nuclear receptor subfamily 2, group F member 2 (Nr2fl); apolipoprotein regulatory protein 1; member of the COUP-family of steroid hormone orphan receptors; liver, lung, kidney; X76653
			Transcription Factors
4	3	0.016	Sine oculis-related homeobox 1 homologue (Drosophila) (Six]); AREC3; expressed in many cell-types during development; ET61028
9	7	0.003	cAMP responsive element binding protein 1 (Crebl); a mediator of cAMP responsive transcriptional regulation; ubiquitous; X67719

LTCR*	STCR*	*P*	GENE
>100	>100	<0.001	Reticuloendotheliosis (Red; c-rel: member of the Rel/nuclear factor (NF)-kappaB family of transcriptional factors; ubiquitous; X15842
>100	>100	<0.001	E4F transcription factor 1 (E4fl); DNA binding transcription factor; ubiquitous; X76858
4	4	0.026	Forkhead box CZ (Foxc2); transcription factor; hepatocytes; X74040
11	11	0.001	Homeo box A9 (Hoxa9); transcription factor; embryogenesis; M28449
>100	>100	0.003	Homeo box msh-like 1 (Msxl); transcription factor; early stage of eye developmental regulation in embryo; embryogenesis; X59251
2	3	0.003	Inhibitor of DNA binding 4 (Idb4); dominant negative regulator of bHLH transcription factors; myogenesis, neurogenesis D83and haematopoiesis; liver and elsewhere; X75018
>100	>100	0.010	Myogen factor 5 (Myf5); transcription factor; embryonic liver and heart; X56182
6	8	0.003	Nuclear transcription factor-Y alpha (Nfya); CHAT-box DNA binding protein subunit A; involved in activation of many hepatic genes; ubiquitous; X55315
3	3	0.018	Paired box gene 2 (Pax2); Pax2 transcription factor; developing embryo excretory and CNS; X55781
12	13	0.003	RE1-silencing transcription factor (Rest); transcription factor; represses expression of neuronal genes; many nonneuronal cells and tissues; U13878
>100	>100	0.002	Sine oculis-related homeobox 1 homolog (Drosophila) (Sixl); homeobox; development of limb tendons; skeletal and smooth muscle cells; X80339
>100	>100	0.005	SRY-box containing gene 12 (Soxl2); transcription factor; Sox family plays important role in development; developing embryos; ET62446
xxx			
2	3	0.032	T-box 4 (*Tbx4*); DNA binding domain putative transcription factor; putative roll in inductive interactions during embryogenesis; embryonic development; ET62078
>100	>100	0.009	Trans-acting transcription factor 1 (*Sp1*); transcription factor; component of some hepatic glucose response elements, ubiquitous; X60136
>100	>100	0.024	Transcription elongation factor A 1 (*Tceal*); transcription elongation factor; liver; D00925
14	12	<0.001	Yes-associated protein, 65 kDa (*Yap*); transcription activator; ubiquitous; X80508
10	10	<0.001	Zinc forger protein 37 (*Zfp37*); putative transcription factor; peroxisome proliferator responsive; liver; X89264
>100	>100	0.009	Zinc finger protein 61 (*Zfp61*); putative transcription factor; liver, elsewhere; L28167
			<u>Translation / Splicing / RNA Processing Factors</u>
7	7	0.001	Cytoplasmic polyadenylation element binding protein (*Cpeb*);

LTCR*	STCR*	*P*	GENE
			RNA binding protein that promotes polyadenylation and translational activation; ubiquitous; Y08260
4	4	0.011	Eukaryotic translation initiation factor 1A (*Eifla*); ubiquitous; U28419
>100	>100	<0.001	Ribosomal protein L32, pseudogene (*Rp132-ps*); ubiquitous; K02060
>100	>100	0.000	Ribosomal protein L7 (*Rp17*); incorporated into 60 S subunit; ubiquitous; X57960
18	13	0.001	Signal recognition particle 9 kDa (*Srp9*); synthesis and translocation of membrane and secreted proteins into the endoplasmic reticulum; ubiquitous; X78304
>100	>100	0.004	Splicing factor arginine/serine-rich 3 (*Sfrs3*); splicing factor belonging to the highly conserved family of SR proteins; regulation of constitutive and alternative splicing; ubiquitous; X91656
			Chromatin Structure
4	5	0.009	Chromobox homologue (Drosophila HP1beta) (*Cbx*); modifs chromatin heritably activating or silencing genes; ubiquitous during development; X56690
>100	>100	0.028	Histone H1 subtype a (H1e); chromatin structure; ubiquitous; L04141
>100	>100	<0,001	Histone H1; chromatin structure; ubiquitous; J03482
109	70	<0.001	Histone H1b; chromatin structure; ubiquitous; ET62262
>100	>100	0.024	Histone H2A; chromatin structure; ubiquitous; X16495
4	3	0.030	Histone H2B; chromatin structure; ubiquitous; ET62908
7	8	0.006	Histone H3.1-D (H3-D) and histone H4-D (H4-D); chromatin structure; ubiquitous; U62672
>100	>100	<0.001	Histone H3.2-F (H3-F), histone H2a.1-F (Ma-fl, histone H2b-F (Mb-F); chromatin structure; ubiquitous; U62669
4	4	0.034	HpaII tiny fragments locus 9c (*Htf9c*); structural similarity with yeast nucleic acid-modifying enzymes; activated at. the G1/S transition, and S phase; down-regulated in growth arrested cells; liver (ubiquitous); X56044

*Fold of control

Example 18

CR stimulates the expression of genes enhancing genetic stability and apoptosis

[0094] The accumulation of genetic damage has been postulated to be a cause of aging. Without being limited to any specific mechanism, CR has been postulated to either reduce the rate of accumulation of genetic damage, or to enhance its rate of repair. Both long and short term CR enhanced the expression of numerous genes associated with DNA repair (Table 5). These genes included *Xpa*, which is involved in nucleotide excision DNA repair; and the *Brca2* gene, which is important in DNA double strand break repair and DNA damage induced cell cycle checkpoint activation.

[0095] A theory of aging closely related to the DNA damage theory proposes that the reduction of apoptosis with age, and its restoration with CR plays and important role in aging. This hypothesis proposes that the accumulation of damaged cells with age contributes to aging itself and to the onset of the diseases of aging. Long and short term CR greatly enhanced the expression of a number of genes which choreograph the progression of a cell through the apoptotic pathway (Table 5). These genes included *Casp1*, *Casp3*, *Bax*, and *Bcl2* which code for key components of the apoptotic pathway.

Table 5. Genetic stability and apoptosis

LTCR*	STCR*	*P*	GENE
			DNA/Replication/Repair
9	8	<0.001	Antigenic determinant of rec-A protein (*Kin*); Kin17; DNA-binding nuclear protein upregulated in response to UV and ionizing radiation; accumulated in the nucleus of proliferating cells; ubiquitous; X58472
>100	>100	0.001	Breast cancer 2 (*Brca2*); DNA double-strand break repair and DNA damage-induced cell-cycle checkpoint activation; ubiquitous; ET62746
3	3	0.029	DNA primase p49 subunit (*Prim*); DNA replication; liver (ubiquitous); X74351
6	5	0.009	Mut L homologue 1 (E. Coli) (*M1h1*); transcription-coupled nucleotide excision repair; cell cycle checkpoint control; ubiquitous; ET63479
3	3	0.025	Xeroderma pigmentosum complementation group A (*Xpa*); nucleotide excision DNA repair; ubiquitous; X7435
			Apoptosis
>100	>100	0.001	B-cell leukemia/lymphoma 2 (*Bcl2*); suppresses apoptosis by controlling mitochondrial membrane permeability; many cells and tissues; L31532
>100	>100	<0.001	Bcl2-associated X protein (*Bax*); pro-apoptotic activity; can form channels in lipid membranes; many cells and tissues; L22472
5	4	0.033	Caspase 1 (*Casp1*); cysteine protease mediator of apoptosis; ubiquitous; U04269
2	3	0.000	Caspase 3 (*Casp3*); cysteine protease mediator of apoptosis; ubiquitous; ET63241
3	4	0.005	Cyclin G (*Ccng*); augments apoptosis; target gene of P53; liver, elsewhere; Z37110
>100	>100	<0.001	Fused toes (*Fts*); a gene related to ubiquitin-conjugating enzymes; suggested role in apoptosis during development; expression distribution poorly defined; X71978
22	21	<0.001	P53 specific ubiquitin ligase 2 (*Mdm2*); promotes ubiquitination and proteaesome degradation of p53; inactivation by stress causes cell cycle arrest and apoptosis; liver, elsewhere; X58876
>100	>100	<0.001	RNA-dependent EIF-2 alpha kinase; double-stranded RNA-

LTCR*	STCR*	*P*	GENE
			dependent protein kinase; key mediator of antiviral effects of interferon; ubiquitous; ET61211
>100	>100	0.009	Tumor necrosis factor (*Tnf*); Proapoptotic factor in liver; X02611

*Fold of control

Example 19

C2 Activation of Genes of the Enteric Nervous System

[0096] The liver is a highly innervated organ. This innervation includes elements of the enteric nervous system, as well as sympathetic innervation in the small arteries of the hepatic mesentery. This nervous innervation is essential to the activity of the liver. Nervous innervation has a role in the release of glucose by hepatocytes in response to insulin. As shown in Table 6, long and short term CR activated the expression of a large number of genes associated with the membrane receptor signaling, including membrane receptors for protein and small molecule neurotransmitters, and for cell growth and maintenance factors. CR induced the expression of genes for both phosphatases and kinases involved in signaling by these receptors. CR also induced the expression of four neuronal tissue specific transcription factors (Table 6).

[0097] CR enhanced the ability of liver neurons to transduce and respond to nervous system signaling. Eight genes for membrane channels were induced, including genes for sodium, potassium, and water channels (Table 6). Also induced were a number of integral membrane proteins such as proteolipid protein and cadherin 8, as well as the products of 5 genes for molecular motors which are probably involved in neural plasticity and remodeling. These proteins included 4 members of the dynein, axon, heavy chain family. Our results are consistent with the idea that CR increases the remodeling and activity of hepatic nerves after only 4 weeks.

Table 6. Neuronal Cell Specific Genes

LTCR*	STCR*	*P*	GENE
			Signal Transduction
19	18	0.001	5-hydroxytryptamine (serotonin) receptor 1E beta (*Htrleb*); G-protein-coupled receptor; CNS; Z14224
>100	>100	<0.001	Activin A receptor, type 1 B (*Acvr1b*); limb development; embryo brain, dorsal root ganglia, spinal cord, vibrissae, elsewhere; Z31663
5	5	0.005	Ankyin 3 (*Ank3*); implicated in Na(+) channel clustering and activity; neuronal axons, wide distribution; ET62740
3	3	0.022	Bone morphogenetic protein receptor, type 1B (*Bmpr1b*);

LTCR*	STCR*	*P*	GENE
			activin receptor-like kinase-6; serine-threonine kinase; CNS, muscle, blood vessels, others; Z23143
5	6	0.004	Discs, large homologue 1 (Drosophila) (*Dlgh1*); role in localization and function of glutamate receptors and K(+) channels; neurons, epithelial cells; ET61665
67	70	0.001	Eph receptor A7 (*Epa7*); developmental kinase 1; member of receptor tyrosine kinase family; brain, testes and spleen; X79082
>100	>100	0.001	Fibroblast growth factor 9 (*Fgf9*); autocrine/paracrine growth factor; embryonic neural cell differentiation; adult and developing neuronal cells, epithelial cells, others; U33535
14	15	<0.001	Fibroblast growth factor homologous factor 1 (*Fgf1*); nervous system development and function; highest in brain and skeletal muscle; U66201
17	19	0.003	G-protein-coupled receptor, family C, group 1, member H (*Gprc1h*); glutamate receptor, metabotropic 8; CNS, filial cells, retina, olfactory bulb, stellate/basket cells; U17252
28	29	<0.001	Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 3 (*Gabrb3*); links binding of GABA to inhibitory chloride flux; CNS; U14420
12	11	<0.001	Glutamate receptor, ionotropic, kainate 1,(*Grik1*); CNS; X66118
>100	>100	0.007	Gonadotropin releasing hormone receptor (*Gnrhr*); G-protein-coupled receptor; activates MAPK cascades; brain, anterior pituitary, reproductive organs; L28756
4	3	0.018	H6 homeo box 2 (*Hmx2*); specification of neuronal cells; developing CNS; S80989
>100	>100	0.001	Histamine receptor H1 (*Hrh1*); coupled to phosphoinositide turnover-calcium mobilization signaling; regulates IGF-I expression, cell proliferation, neural function; neurons, liver, elsewhere; D50095
64	73	<0.001	Neuropeptide Y receptor Y6 (*Npy6r*); regulates energy balance through its orexigenic, antithermogenic, and insulin secretagogue actions; neurons, vascular smooth muscle cells; U58367
>100	>100	<0.001	paired-Ig-like receptor A1 (*Piral*); activating receptor on B lymphocytes; dendritic and myeloid-linage cells; ET62839
4	4	0.00	Preproglucagon (*Gcg*); glucagon-like peptides I and II; neuropeptide; CNS, pancreatic alpha cells, ileum; Z46845,
>100	>100	0.013	Protein kinase, cGMP-dependent, type II (*Prkg2*); signal transduction; brain, kidney, small intestine, colon; L12460
>100	>100	0.001	protein tyrosine phosphatase, receptor type, M (*Ptprm*); expressed in capillaries in developing neural tissue, lung; X58287
>100	>100	<0.001	Relaxin precursor (*Rln*); insulin gene family; remodeling of collagen; brain, uterus, prostate, pancreas and kidney; Z27088
>100	>100	<0.001	Ryanodine receptor 3 (*Ryr3*); intracellular Ca2+ channels;

LTCR*	STCR*	*P*	GENE
			neurons, skeletal and smooth muscle; ET61090
			Neuronal Tissue Specific Transcription Factors
>100	>100	<0.001	Atonal homologue 5 (Drosophila) (*Atoh 5*); neurogenin 3; transcription factor; neuroD-related bHLH protein; CNS; U76208
19	18	0.003	Embigin (*Emb*); DNA-binding transcription factor; class VI POU domain; CNS; D13801
>100	>100	0.026	Paired box gene 6 (*Pax6*); transcription factor; development of CNS, eye; X63963
>100	>100	<0.001	Zinc finger protein 2 (*Zfp2*); Mkr-2; differentiation and/or maintenance of neurons; central and peripheral neurons; Y00850
			Channels
4	3	0.007	Aquaporin 4 (*Aqp4*); allows water and small solutes through plasma membrane; brain and other tissues; U48397
5	6	0.004	Discs, large homologue 1 (Drosophila) (*Dlgh1*); localization and function of glutamate receptors and K(+) channels; neural synapses; ET61665
22	25	0.001	Gap junction membrane channel protein beta 6 (*Gjb6*); connexin 30; forms transmembranous gap junction channels between adjacent cells; brain, skin; ET63385
11	11	0.001	K+ channel beta-subunit, ion channel; brain and kidney; X97281
14	16	0.001	Potassium inwardly-rectifying channel, subfamily J, member 6 (*Kcnj6*); neurons; ET61642
8	8	0.005	Potassium voltage gated channel, shaker related subfamily, member 2 (*Kcna2*); T cells, myelinating Schwann cells; M30440
27	28	<0.001	Sodium channel 27; brain; L42340
11	11	<0.001	Sodium channel, type X, alpha polypeptide (*Scn10a*); brain, unmyelinated axons; Y09108
			Molecular Motors
2	2	0.004	Dilute lethal-20J; Class-V myosin; vesicular membrane trafficking; transport of endoplasmic reticulum vesicles in neurons; M33467
7	8	0.001	Dynein, axon, heavy chain 1 (*Dnahc1*); dyneins are molecular motors that drive the beating of cilia and flagella; brain, trachea, testis; ET63395
>100	>100	<0.001	Dynein, axon, heavy chain 3 (Dnahc3); brain, trachea, testis; ET63399
5	6	0.013	Dynein, axon, heavy chain 6 (Dnahc6); brain, trachea, testis; ET63402
4	5	0.002	Dynein, axon, heavy chain 9 (Dnahc9); brain, trachea, testis; ET63405
			Cell Surface and Secreted Proteins
>100	>100	0.001	Cadherin 8 (*Cdh8*); adhesion molecule; subdivisions of the early CNS and thymus; ET63017
37	36	<0.001	Glutamic acid decarboxylase, 67 kD; responsible for gamma-aminobutyric acid synthesis; brain, islets; Y12257

LTCR*	STCR*	*P*	GENE
2	2	0.011	Glypican 4 (*Gpc4*); cell surface heparin sulfate proteoglycan; role in regulation of neural cell transition from proliferation to differentiation; neurons; X83577
19	20	<0.001	Neurexophilin 2 (*Nxph2*); neuronal glycoprotein; binds to alpha-neurexins; brain; U56650
13	13	<0.001	Neurotrophin 3 (*Ntf3*); secreted protein; maintenance and plasticity of neurons; enteric neurons, others; X53257
43	41	0.001	Proteolipid protein (*Plp*), main integral protein of myelin; CNS; X07215
4	4	0.043	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E (*Sema3e*); glycoprotein involved in embryonic development; developing neural tubes, lungs, skeletal elements; ET63410
>100	>100	<0.001	Sema domain, seven thrombospondin repeats (type 1 and type 1-like) (*Sema5a*); axonal guidance; early embryogenesis; X97817
			Other Genes
6	7	0.015	Disabled homolog 1 (Drosophila) (*Dab1*); adaptor molecule in neural development; neuronal and hematopoietic cells; ET63156
23	24	<0.001	Galanin (*Gal*); neuropeptide; enhances hepatic glucose production; hepatic nerves and elsewhere; L38580
3	4	0.006	Netrin 1 (*Ntn1*); axon outgrowth-promoting protein; guidance molecule; guides growing axons in development; CNS; U65418
127	129	<0.001	Nucleosome assembly protein 1-like 2 (*Nap112*); *Bpx*; brain; X92352
>100	>100	<0.001	Proteaseome 3 (*Psme3*); Ki antigen; cell proliferation; enhances generation of class I binding peptides; liver, neurons, elsewhere; U60330
58	58	<0.001	UDP_glucuronosyltransferase 8 (*Ugt8*); cerebroside and sulfatide biosynthesis; CNS and peripheral nervous system; X92122

*Fold of control

Example 20

Induction of other liver specific genes by CR

[0098] Of the approximately 200 genes reported to be expressed either liver specifically or ubiquitously, 13 code for cytokines or growth factors; 12 for cell surface receptors; 21 for signal transduction, cell cycle or cell growth related proteins; 4 for nuclear receptors, 20 for transcription factors; 6 for translation, splicing, or RNA processing related factors; and 9 for chromatin structure related genes (Table 4). The overall pattern of genes induced in this group of genes suggests that CR stimulates the growth, remodeling and responsiveness of

liver cells to signaling systems. These results are consistent with those found for neuronal genes, discussed above.

[0099] Both long and short term CR induced the expression of the cell growth factors *Tgfb2, Fgf1, Fgf2, Fgf3, Fgf7, Fgf9m Figf, Inhbb, Inhbb, Inhbe*, and 3 interferon related genes. Likewise, a large number of genes coding for cell cycle regulation were induced by CR. These genes included *Ptpn16, Nek1, Plcgl, Map3k1, Mapk1, Madh5, Wnt10b, Abl*, and others. Without being limited to any specific mechanism, the hypothesis that CR induces cell remodeling and growth of liver cells is further supported by the observation that both long and short term CR very strongly induced the expression of 7 histone genes. In 6 cases, these mRNA levels were induced from undetectable, or nearly undetectable levels. Two other genes which appear to be associated with chromatin structural modification were also strongly induced by CR (*Htf9c* and homologous to Drosophila *Hp1*; Table 4). Further evidence that CR enhances cell division and remodeling is the up regulation of the mRNA for the transferrin receptor, which mediates cellular iron uptake, a process essential for cell growth and division.

[0100] Three receptor mRNAs associated with energy balance were induced by CR. Two of these were for neuropeptide Y receptor Y6 (Table 6) and pancreatic polypeptide receptor 1, and one was for the leptin receptor (Table 4).

Example 21

Global hepatic gene expression profile

[0101] We have tested the hypotheses that CR produces similar effects on gene expression early and late in life by examining the effects of aging and caloric intake on the expression of approximately 12,000 genes and ESTs in the liver of old (27 month-old) and young (7 month-old), control and CR mice, using GeneChip microarrays. We found that CR produced a massive reprogramming of gene expression early and late in life. The patterns of expression induced by CR in young and old mice were highly homologous. Comparison of gene expression in the groups of mice indicated that CR only prevented age related changes in the expression of a few genes. Examination of the genes involved does not support the idea that they have a principle role in the age-retarding effects of CR. Together, the results do not support the idea that CR acts principally to prevent deleterious age related changes in gene expression. Instead, CR induces a highly homologous, major reprogramming of gene expression in animals of all ages.

[0102] The average global hepatic gene expression profile for each group of mice, displayed using GeneSpring 3.0 (Silicon Genetics, San Carlos, CA), is shown in Figure 8. The GeneSpring experiment tree algorithm clustered gene expression in the young and old CR mice together, and separately clustered expression in the young and old control mice together. These results indicate that that the effects of the CR diet on gene expression was significantly greater than the effect of age. Further, these data indicate that CR produced homologous effects on gene expression in the young and old mice.

Table 7. Pairwise comparisons of the global gene expression correlation coefficients for each possible pair of mice.

	Old-CR	Old-Control	Young-CR	Young-Control
Old-CR	0.53 ± 0.02	-0.09 ± 0.02	0.41 ± 0.04	-0.10 ± 0.03
Old-Control		0.28 ± 0.06	-0.11 ± 0.03	0.23 ± 0.02
Young-CR			0.41 ± 0.01	-0.08 ± 0.02
Young-Control				0.22 ± 0.02

*All values average values, ± SD are calculated as the Log (1+ the mRNA level)

[0103] These conclusions are supported by comparison of the correlation coefficients calculated from the expression data for each possible pair of mice in the study (Table 7). Because the mice were genetically identical, infra group values provide a measure of the maximum correlations attainable. Inter group values measure the similarity between groups. Inter group comparisons between young and old CR and control mice indicated that gene expression in all CR mice was highly homologous, regardless of the age of the animals. Likewise, regardless of age, the infra group expression patterns of the control mice were highly homologous. In contrast, there was no infra group correlation between mice in different dietary groups, regardless of age. These data indicate that the number of calories consumed, but not age was the major influence in determining the global patterns of gene expression in these mice. This novel result is further supported by the analysis described below.

[0104] The patterns of gene expression in the mice were further evaluated by successive application of the Venn Diagram Function of GeneSpring 3.0, one way ANOVA, and Fisher's test ($P < 0.05$) to the levels of expression of each gene and expressed sequence tag (EST) in the 4 groups of mice. These operations sorted the genes and ESTs into one of 9 possible

categories (Tables 8A and B). Only statistically significant differences of 2-fold or more are shown. The expression of most genes and ESTs were not affected by either CR (~80% uncharged) or aging (95% unchanged). Of the genes and ESTs which did changed expression among the groups, 5-times as many genes and ESTs changed expression level in response to CR (2456) as changed in response to age (561). Of the genes and ESTs responsive to CR, most (40%) were upregulated in both young and old mice. Two other groups of genes and ESTs were upregulated either in old mice only (28% of the genes that changed expression), or in young mice only (19% of the genes that changed expression). An even smaller number of genes and ESTs were down regulated by the CR diet in young or old mice (13% of the genes that changed expression).

Table 8. The effects of age and diet on gene expression

a. Diet Effect Old

Young (CR/Control)*	Old (CR/Control)* Up**	Unchanged	Down**	Total
Up**	975 (8.1%***)	473 (3.9%)	0	1448
Unchanged	685 (5.7%)	9587 (79.6%)	172 (1.4%)	
Down**	0	105 (0.9%)	46 (0.4%)	151
Total	1660		218	

*Fold change of average mRNA levels of Old/Young mice
**Fold change of 2-fold or greater
***Percent of total genes and ESTs measured in study

b. Age Effect Old

Young (CR/Control)*	CR (Old/Young)* Up**	Unchanged	Down**	Total
Up**	6 (0.05%***)	136 (1.1%)	2 (0%)	144
Unchanged	186 (1.5%)	11482 (95%)	112 (0.9%)	
Down**	1 (0%)	113 (0.9%)	5 (0.04%)	119
Total	193		119	

*Fold change of average mRNA levels of Old/Young mice
**Fold change of 2-fold or greater
***Percent of total genes and ESTs measured in study

Example 22

208 Genes greater in CR in both Young and old

[0105] Three novel conclusions can be. drawn from these data. First, CR induced a substantial age independent reprogramming of gene expression. A large number of genes and ESTs (975) were up regulated by CR in both young and old mice (Table 8A). In this group, 208 were known genes (See Appendix G) All of these known genes were among the group of 340 genes induced in 30 month old mice by both long term CR (LT CR; life long) and short term CR (ST CR; only 4 weeks of CR). This highly reproducible, age independent, responsiveness to CR suggests to us that these genes and ESTs are likely to mediate the life

and health span extending effects of CR. At a minimum, the dietary responsiveness of these genes can be used as a gauge of the effectiveness of other treatments in reproducing the effects of CR on global patterns of gene expression. Further, because 90% of the genes and ESTs induced by lifelong CR (which includes the age independent and age dependent genes and ESTs) can be induced after only 4 weeks of CF, the vast majority of the genetic reprogramming induced by CR can be reproduced rapidly.

Example 23

142 genes Up in young LCR but not in old CR

[0106] There is a second novel conclusion which can be drawn from the results in Table 8A. CR produced some "age dependent" reprogramming of gene expression in both young and old mice. Of the 473 genes and ESTs induced by CR only in young mice, 142 are known genes (Appendix H). These results indicate that this subset of genes was also CR responsive in old mice, but not to sufficient levels that they were distinguished statistically from control expression levels in these studies. Thus, Table 8A overestimates the number of young-specific induced genes by approximately 25%. Of the young-specific genes, 8% are involved in transcriptional regulation; 5% are growth factors, cytokines or hormones; 18% are involved in signal transduction or cell cycle regulation; 14% are involved in embryogenesis and development; 14% are involved in cellular adhesion, or are components of the extracellular matrix or membrane; 7% are channels or ion pumps; 3% are involved in extracellular transport or secretion; 3% are involved in metabolism; 3% in DNA replication, repair or apoptosis; 3% in chromatin structure; 9% in immune function or in the primary response; and 15% are involved in other functions.

Example 24

200 known genes greater in old CR but not in young CR

[0107] Of the 685 genes and ESTs induced by CR in old mice, the identity of 200 are known (Table 8A); (Appendix I). Of these, 122 (61%) previously were shown to be induced by ST-CR in old mice. Thus, the majority are rapidly responsive to CR. Of the remaining 78 genes, approximately 12% are transcriptional regulators; 8% are growth factor, cytokines or hormones; 13% are involved in signal transduction or cell cycle regulation; 11 % are involved in embryogenesis and development; 10% are involved in cellular adhesion, or are components of the extracellular matrix or membrane; 4% are channels or ion pumps; 4% are involved in extracellular transport or secretion; 3% are involved in metabolism; 3% in DNA

replication, repair or apoptosis; 2% in chromatin structure; 3% in immune function or in the primary response; 2% in translation, splicing or RNA processing; 2% are cell surface receptors; and 23% are involved in other functions.

[0108] The proportion of genes involved in each functional category above are remarkably similar. Further, many of the genes induced by CR in young mice were members of similar gene families or were structurally or functionally related to genes induced only in old mice. These similarities suggest that CR has highly homologous age specific effects. It is less likely that the relative proportion of genes falling into each category, and the identity of these genes is an artifact of the probes present on the chip. Firstly, all of the results are statistically significant. Second, the genomic profiles produced in several drug studies were strikingly different from those found here as to the identity of the genes affected, and their functional categories (data not shown). Together, these results indicate that CR has a robust, pervasive, and highly homologous effect in both young and old mice. It induced the expression of a substantial group of genes involved in a wide variety of cellular functions.

[0109] A commonly expressed view in the literature of CR and aging assumes tacitly or explicitly that CR acts by preventing deleterious, age related changes in gene expression. This view is shown schematically in Figure 9. This hypothesis assumes that prevention of age related changes in gene expression underlies the health and lifespan extending effects of CR. During aging, some genes become over expressed or under expressed relative to their levels in young animals (lower and upper lines, Figure 9). Some of these deviations are assumed to be deleterious. Preferably, no changes would change with time, and aging would either not occur or occur more slowly (center line, Figure 9). In this view, CR should wholly or partially return over- or under-expressed genes to their youthful levels (arrows, Figure 9). Although the reasoning is circular, some have said that if CR changes the expression of a gene toward the center line in the figure, it restored youthful levels of expression. We have analyzed the results of the studies reported here to evaluate this hypothesis further.

[0110] Of the approximately 12,000 genes and ESTs examined, aging of control mice increased the expression of 257 genes and ESTs, and decreased expression of 191 genes and ESTs (Figure 9). Long term CR wholly or partially, reversed or prevented 55 of the increases and 70 of the decreases. Short term CR reversed 45 of the increases and 59 of the decreases in gene expression. Long term and short term CR both acted to reverse or prevent 23 of the increases and 41 of the decreases. Thus, long term CR actually prevented the increased expression of only 32 genes and ESTs and the decreased expression of only 29 genes and ESTs. It is likely that the number of ESTs in each class overestimates the number of

authentic genes in each category. First, the genes and ESTs which responded to CR in only 4 weeks are likely a subset of the genes and ESTs which respond acutely to CR. We have not yet examined longer times on the domain of genes responsive to acute CR. Some genes may be "slow changers" in response to acute CR. Second, we have found that many of the known genes present on these chips are redundant (e.g., multiple immunoglobulin genes of each class and T cell receptor genes, cloned chromosome breakpoints representing parts of two genes, uncharacterized chromosome regions, uninvestigated, unpublished cDNA sequences, etc.). For example, of the 23 genes and ESTs reduced to baseline expression levels only by LT-CR, 12 were known genes (Table 9). Of the 27 genes and ESTs which were decreased in expression by age and returned to baseline expression only by LT-CR, only 13 were from known genes (Table 10).

[0111] Of the 12 genes prevented from increasing with age by CR, few are involved in signal transduction. Rather, 6 are involved in immune system function, particularly in macrophage differentiation, proliferation, apoptosis, and activity. Of these, platelet-activating factor acetylhydrolase activity reduces plasma platelet activating factor mRNA levels. Platelet activating factor is a potent pro-inflammatory autacoid with diverse physiological and pathological actions. It does not seem likely that the return of these genes to baseline expression levels is due to a general reduction in inflammation, stress, or immune activity. In a previous study, we found that 61 immune system genes, including 6 primary response genes, and an additional 9 apoptotic genes were up regulated by both LT- and ST-CR in the liver of mice. Similar considerations apply to the other 6 genes in this group, and to the genes prevented from decreasing with age (Table 10). One can speculate about why reduction in the expression of the relatively few immune system specific, acute phase response genes and other genes listed in Table 9, or enhanced expression of the 13 immune system, and neuron or liver specific genes in Table 10 might be important in reducing the rate of aging. However, with few exceptions, very similar genes, and in some cases closely related family members of the genes in these lists are present in the group of 340 known genes induced by both LT- and ST-CR. Thus, it seems intuitively and statistically much more likely that the massive reprogramming of gene expression induced by CR (Tables 9 and 10) is responsible for the increase in life and health span induced by CR. The genes prevented from increasing and decreasing with age (Tables 9 and 10) seem much more likely to be the result, rather than the cause of these effects.

[0112] In summary, the studies presented here show that a major effect of CR is to massively (more than 10% of the genes and ESTs investigated) reprogram gene expression to

a new pattern associated with slower aging and delayed onset of age-related diseases. This reprogramming includes age independent induction of a relatively large group of genes and ESTs, as well as induction of smaller groups of genes age dependently. Further, we found that age related changes in gene expression are relatively rare. Even rarer are instances in which life long CR prevents these changes. The rarity of such genes, and their identity suggest to us that they do not play a major role in the physiological effects of CR. The large and rapid response induced by CR on total liver gene expression suggests that major, systemic regulators of gene expression are altered by CR. Study of the regulation of a number of these genes should yield the identity of the regulators, and reveal how they are influenced by CR.

Table 9: rRNAs increased by age and returned to control levels by LT-CR

GenBank	Phenotype
	Immune System
AF018268	Apoptosis inhibitory 6 (Api6); a member of macrophage scavenger receptor cysteine rich domain superfamily; inhibits apoptosis of a variety of cell types; secrete specifically by macrophages
M13018	Cysteine rich intestinal protein (Crip); double zinc finger protein; expression change with acute liver injury (cellular damage); may function in cell proliferation differentiation or turnover; high expression in immune cells, low in liver
J04596	GRO1 oncogene (Gro1); encodes a cytokine; mediator of inflammatory and immune responses; also called melanoma growth-stimulatory activity; cell cycle regulator platelets
L20315	Macrophage expressed gene 1 (Mpeg1 or Mpg-1); increased when marine fetal live hematopoietic progenitor cells induced to differentiate into macrophages; high level in macrophages, moderate levels in certain myelomonocytic cell lines
U34277	Phospholipase A2 group VII, platelet-activating factor acetylhydrolase, plasm (Pla2g7); secreted phospholipase A2 which modifies the pro-inflammatory platelet activating factor (PAF) to yield the biologically inactive lyso-PAF; regulates baseline circulating PAF levels and may be critical in resolving inflammation; high PAF is predictor of heart disease; liver macrophages
L27990	Sjogren syndrome antigen A1 (Ssal); Ro52; stress response gene; ribonucleoprotein macrophages
	Ubiquitous
D86729	Heterogeneous nuclear ribonucleoprotein A1 (Hnrpal); ribonucleoprotein, RN processing; early down-regulation of this gene contributes to the cytotoxicity of the topoisomerase inhibitors that induce DNA cleavage; ubiquitous

GenBank	Phenotype
	Immune System
U50850	Retinoblastoma-like 2 (Rb12); p130; transcriptional cell cycle repression through C phase (controls cyclin A, cdc 25G and cdc2 genes); tumor suppressor gene; express independently of retinoblastoma gene; expressed in embryo and ubiquitously in adults
U34042	Tolloid-like (Tll), an alternatively spliced product of the bone mozphogenic protein gene; metalloprotease purified from extracts capable of inducing ectopic bo formation; ubiquitous
	Liver Specific
U60438	Serum amyloid A protein isoform 2 (Saa2); encodes an acute-phase reactant serum protein; liver
	Not Reported in Liver
M27501	Protamine 2 (Prm2); compacting chromatin; expressed in postmitotic male germ cell during late stages of spermatogenesis
U52433	Tubby (Tub); mutation in the tub gene causes maturity-onset obesity; adipocyte storage increased by 5-6 fold, insulin resistance; mutant mice have retinal a cochlear degeneration; gene function unknown; brain, hypothalamus, cochlea, retin.

Table 10 mR:VAs decreased by age and returned to control levels by LT-CR

GenBank	Phenotype
	Immune System
M30903	B lymphocyte kinase (Blk); src-family protein tyrosine kinase; plays important role in B-cell development/activation and immune responses; B-lineage cells
U43384	Cytochrome b-245, beta polypeptide (Cybb, cytochrome b558); integral component of the microbicidal oxidase electron transport chain of phagocytic cells, respiratory burst oxidase; phagocytes
U10871	Mitogen activated protein kinase 14 (Mapk14); signal transduction, stimulate phosphorylation of transcription factors; major upstream activator of MAPKAP kinas 2; hematopoietic stem cells
222649	Myeloproliferative leukemia virus oncogene (Mpl); Member of hematopoietic cytokine receptor family, cell cycle regulator, induces proliferation and differentiation of hematopoietic cell lines; hematopoietic precursor cells, platelets and megakaryocytes
Y07521	Potassium voltage gated channel, Shaw-related subfamily member 1 (Kcncl) potassium channels with properties of delayed rectifiers; nervous system, skeletal system, T lymphocytes
U87456	Flavin-containing monooxygenase 1 (Fmol); xenobiotic metabolism; highly expressed in liver, lung, kidney, lower expressed in heart, spleen, testis, brain

GenBank	Phenotype
	Immune System
U40189	Pancreatic polypeptide receptor 1 (Ppyrl), neuropeptide Y receptor, peptide Y receptor; G-protein-coupled receptor; liver, gastrointestinal tract, prostate, neurons endocrine cells
	Neuron Specific
U16297	Cytochrome b-561 (Cyb561); electron transfer protein unique to neuroendocrin secretory vesicles; vectoral transmembrane electron transport; brain
D50032	Trans-golgi network protein 2 (Ttgn2); integral membrane protein localized to the trans-Golgi network; involved in the budding of exocytic transport vesicles; brain neurons
	Liver Specific/Ubiquitous
D82019	Basigin (Bsg), CD147, neurothelin; membrane glycoprotein, immunoglobulin superfamily, homology to MHCs, acts as an adhesion molecule or a receptor, near: network formation and tumor progression; embryo, liver and other organs
L38990	Glucokinase (Gk), key glycolytic enzyme; liver
U50631	Heat-responsive protein 12 (Hrsp12); heat-responsive, phosphorylated protein sequence simularity to Hsp70; liver, kidney
U39818	Tuberous sclerosis 2 (Tsc2); mutationally inactivated in some families with tuberoi sclerosis; encodes a large, membrane-associated GTPase activating protein (GA tuberlin); may have a key role in the regulation of cellular growth; ubiquitous

Example 25

Gene expression in STZ-diabetic mice

[0113] Streptozotocin (STZ) induces diabetes. Mice receiving three treatments with STZ were diabetic for about 4 weeks. Diabetes reduces insulin levels to almost zero. CR has a similar effect in that it lowers insulin levels, although not as low as in STZ-treated animals. Also, while CR lengthens life span, STZ has the opposite effect and shortens life span.

[0114] Figure 10 shows pairwise comparison of global gene expression correlation coefficients for each possible mouse pair. The results indicate that hepatic gene expression is very different between young CF, young control and STZ-diabetic mice. Figure 11 presents a visual profile which shows that the pattern of gene expression in the three groups is dissimilar. In conclusion, lowering insulin in the pathological way found in serious diabetes is insufficient to produce the gene expression profile or the life span effects observed with CR.

Example 26

Gene expression in aminmanidine treated mice

[0115] Aminoguanidine is believed to retard aging by preventing cross linking of protein initiated by the aldehyde form of glucose. However, mice fed aminoguanidine exhibited little or no effect on life span. However, a large effect on gene expression was observed (Figure 12). Gene expression for aminoguanidine treated mice did not correlate with either old CR or old control. A visual representation of this finding is shown in Figure 13. In conclusion, although aminoguanidine has little effect on aging in mice, major differences in gene expression are observed. These effects are not like those of CR, and this is consistent with the absence of a strong effect on the life span of mice.

Example 27

[0116] To determine whether certain interventions mimic calorie restriction in mice, the following groups of mice are prepared.

[0117] Group 1: Controls

[0118] Group 2: Troglitazone (synthetic proposed calorie restriction mimetic drug that lowers insulin levels in rats and mice, lowers blood pressure and triglycerides, inhibits free radicals, increases mitochondria) mass, and doesn't seem to change food intake in rodents): treatment starts at 10 months

[0119] Group 3: IGF 1 (natural proposed calorie restriction mimetic hormone that lowers both insulin and glucose levels and which may be directly involved in the basic mechanisms of aging; has rejuvenating effects on immune, muscular, and other systems): treatment starts at 12 months

[0120] Group 4: ALT 711 (or other AGE breaking agent: proposed calorie restriction mimetic that acts by reversing the effects of elevated glucose levels as they occur or after they occur, rather than by reducing glucose levels): treatment starts at 18 months.

[0121] Animals in all groups will receive the same, known amount of food throughout the study.

[0122] Troglitazone and IGF-1 doses will be chosen to set glucose and insulin levels in the range for young or preferably calorie restricted animals. Glucose and insulin will be measured but not controlled in the control and ALT-711 groups. Troglitazone will be supplied at a dose of ~0.2% of the diet (standard for troglitazone studies for other purposes). Similarly, ALT-711 will be incorporated into the diet. A low (non toxic) level of ALT-711 is used that will remain constant over time.

[0123] It is assumed that IGF-1 will be supplied by injection (3 times per week, minimum) unless a continuous delivery method can be arranged. The preferred dosage method is implantation of non dividing IGF-1 secreting cells, to attain steady IGF-1 levels, and if possible, this will be done. If this is not possible, IGF-1 will be obtained as a gift from Genentech or another manufacturer. Other possible alternatives to injection are: osmotic minipump; injection of IGF-1 into subcutaneous slow release reservoirs; infusion by means of minipumps used by Celtrix; use of skin patches that allow slow release to the body.

[0124] There will be 60 animals in each longevity testing group (LTG). Each LTG will be accompanied by another set of, on average, 40 similarly treated animals, which will be set aside for sacrifice to permit biochemical assays and histological documentation of the condition of the animals at fixed ages (sacrifice group, SG). In the case of the IGF-1 and troglitazone groups, some animals will be earmarked for pilot dose finding experiments in a manner that will allow the average SG size to remain at 40, as described below. The groups earmarked for dose verification will be referred to as the pilot dose groups, or PDGs.

[0125] For troglitazone, about a 2 month supply of each of three troglitazone diets (containing 0.1%, 0.2%, or 0.3% troglitazone) will be initially ordered. The main 0.2°,% troglitazone dose will be tested on a small pilot mouse population before committing the troglitazone group proper to this dose. If 0.2% troglitazone is not found to yield the expected changes in circulating insulin after 2 weeks on the 0.2% troglitazone, the diet will be changed to the more appropriate dose diet at that time and verified on a second small pilot mouse population.

[0126] Similarly, some animals will be used for IGF-1 injection pilot experiments to determine the proper starting dose.

[0127] At age 12 months: Sacrifice 3 animals/SG to obtain common baseline group of 12 animals to be compared to all subsequent results. This is the middle aged universal control group. All subsequent data can be compared to the results for this pooled group.

[0128] At age 12.5 months: Begin the IGF-1 PDG with 7 mice given the best estimated dose of IGF-1. Sacrifice two weeks later for determination of insulin and glucose levels. Begin a verification/second trial dose of IGF-1 at 13 months, 1 week of age, and sacrifice this second PDG at 13 months, 3 weeks of age. Assuming the assays for insulin and glucose can be completed in 1 week, this regimen will allow the final dose for the LTG to be determined prior to age 14 months. Similarly, at 12.5 months, place 7 mice on the 0.2% troglitazone diet. Two weeks later, sacrifice and assay for insulin and glucose. Begin adjusted dose or verification dose group at 13 months, 1 week and sacrifice after two weeks.

[0129] At age 14 months: Begin troglitazone and IGF-1 at the experimentally-determined or estimated optimal doses for each.

[0130] At age 15 months: Sacrifice six animals from the IGF-1 and troglitazone SGs for determinations of glucose, insulin, and all other endpoints involved in the study. If necessary, adjust the IGF-1 dose again (both in the LTG and the untapped portion of the IGF-1 SG) and/or order diet with a modified troglitazone content. Sacrifice three animals each from the SGs for the controls and the ALT-11 groups and pool to create a common group of six animals for comparison to the IGF-l and troglitazone groups.

[0131] At age 18 months: same as at 15 months, but use 7 mice/SG for IGF-1 and troglitazone and 4 mice/SG for the control and for the ALT-711 group. Begin the ALT-711 groups on ALT-711 immediately after this sampling.

At around 27 months (~24 30 months): Sample all remaining surviving SG mice.

[0133] If the total initial numbers of mice in the sacrifice groups for treatments 1, 2, 3, and 4 are 30, 50, 50, and 30, respectively, then if there were no mortality in any of these groups, there would be 20 animals left in each SG at the time of final sampling. But if we assume that only 1/3 of this number will be alive, then about 7 animals will remain to be sampled at the final sample time, or about the minimum required for statistical significance. If the mean survival rate at 27 month is over 73%, the 27 month end point may be postponed to a greater age.

[0134] In addition to other biochemical markers, assays may include:

heart and thymus volume and histology;

autoantibody titer;

T and B cell characteristics;

protein or albumin concentration in bladder urine at sacrifice;

molecular glycation indices;

protein carbonyl content or other free radical/oxidation indices;

and incidence of neoplasia, esp. of prostate and breast.

APPENDIX A

P	CR	std	CON	std	SW	std	GenBank	Description	Location
NOT REPORTED IN LIVER NERVOUS TISSUE									
0.046	0.88	0.79	0.00	0.00	1.20	0.18	L34676	X11 protein gene; X11 protein binds amyloid precursor protein; receptor trafficking; + I543 may regulate the processing of amyloid precursor protein to the amyloid beta peptide	Neurons
0.006	0.95	0.27	0.28	0.27	1.23	0.11	U65418	Netrin-1; axon outgrowth-promoting protein; guidance molecule; guides growing axons in development	CNS
<0.001	1.10	0.02	0.00	0.00	1.09	0.19	X97817	Semaphorin F; involved in axonal guidance	Early embryogenesis
<0.001	1.02	0.01	0.04	0.08	1.06	0.09	L38580	Galanin; a neuropeptide; enhances hepatic glucose production; present in hepatic nerves	Released by hepatic nerves; CNS and peripheral organs including pituitary, pancreas, small and large intestine, adrenal gland, lung, tongue, testes, ovary-fallopian tubes, and uterus; not detectable in heart, liver, kidney, urinary bladder or skeletal muscle

P	CR	std	CON	std	SW	std	GenBank	Description	Location
<0.001	1.14	0.16	0.02	0.03	1.14	0.16	X92122	UDP-galactose ceramide-galactosyl transferase; key enzyme in cerebroside and sulfatide biosynthesis; glycosphingolipids; most abundant in myelin	CNS and peripheral nervous system; tissue distribution poorly characterized
<0.001	1.10	0.10	0.06	0.10	1.16	0.18	U56650	Neurexophilin 2 (Nxph-2);+I20 neuronal glycoprotein; binds to alpha-neurexins	Brain
<0.001	0.99	0.22	0.04	0.06	1.04	0.06	L42340	Sodium channel 27	Brain; tissue distribution and protein poorly characterized
<0.001	1.16	0.27	0.03	0.06	1.11	0.11	X61449	Brain expressed anonymous cDNA	Brain; expression poorly characterized
<0.001	1.13	0.17	0.01	0.02	1.15	0.20	X92352	Bpx, strong homology to genes encoding nucleosome assembly proteins; poorly characterized	Brain; tissue distribution poorly characterized
0.001	1.29	0.29	0.03	0.05	1.22	0.22	X07215	PLP; proteolipid protein, main integral protein of the myelin	CNS
<0.001	1.17	0.19	0.03	0.06	1.16	0.25	Y12257	Glutamic acid decarboxylase 67 kD.	Brain, islets; isozyme of liver form
0.001	1.36	0.15	0.00	0.00	1.09	0.35	ET63017	Cadherin-8, adhesion molecule	MCad8 expression is restricted to particular subdivisions of the early central nervous system (CNS) and to the thymus
0.001	1.19	0.35	0.09	0.15	1.43	0.12	ET61642	Inward rectifier potassium channel 2 (GIRK2)	Neurons

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	1.18	0.25	0.11	0.19	1.16	0.21	X97281	K + channel beta-subunit, ion channel	Brain and Kidney
<0.001 Labell	1.06	0.07	0.10	0.12	1.08	0.08	Y09108	SNS-TTXi sodium channel, ion channel; small-diameter sensory neurons associated with unmyelinated axons express a tetrodotoxin-insensitive (TTXi) voltage-gated sodium channel (VGSC); may play an important role in the transmission of nociceptive information to the spinal cord	Brain
0.005	1.26	0.03	0.15	0.26	1.16	0.39	M30440	Potassium channel gene (MK2); Shaker subfamily	T cells; myelinating Schwann cells
0.018	1.78	0.50	0.40	0.46	1.10	0.21	S80989	NK-related homeobox gene (Nkx-5.2); cell type specification of neuronal cells	Developing CNS and ear in E13.5 embryos; cell type specification of neuronal cells
0.011	1.00	0.21	0.50	0.24	1.22	0.13	X83577	K-glypican; cell surface heparin sulfate proteoglycan; suggested role in regulating cell cycle progression during the transition of neural cells from proliferation to differentiation.	In embryo major sites are tubular epithelial cells in kidney and proliferating neuroepithelial cells in brain; neurons

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.044	1.12	0.26	0.61	0.27	1.32	0.28	U36757	Thrombin receptor (PAR-1); transmembrane G-protein coupled receptor; activated by serine protease cleavage; thrombin is a serine protease generated by the activation of the blood coagulation cascade following vessel injury; thrombin acts as a mitogen, apoptosis inducer and regulator of inflammation	Blood, platelets, monocytes; endothelial cells; cardiomyocytes; neuronal and glial cells
0.004	1.02	0.02	0.53	0.17	1.02	0.12	M33467	Dilute lethal-20J; Class-V myosin; unique type of myosin motor; role in vesicular membrane traffic through actin rich regions of the cytoplasm; transport endoplasmic reticulum vesicles in neurons and pigment granules in melanocytes	Adult germ line cells; early embryo; oocyte during oogenesis; enriched in brain; neurons; melanocytes
0.000	1.31	0.06	0.00	0.00	0.98	0.06	ET62839	Immunoglobulin-like receptor (PIRA1); activating receptor on murine B lymphocytes; dendritic cells; myeloid-linage cells.	B lymphocytes; dendritic cells, myeloid-linage cells.
0.015	1.14	0.15	0.20	0.20	1.36	0.55	ET63156	Mouse homolog of the Drosophila Disabled (Dab) protein; MDab217; an adaptor molecule functioning in neural development.	Neuronal and hematopoietic cell lines; growing nerves of embryonic mice

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	1.21	0.21	0.05	0.09	1.37	0.36	ET63385	Connexin30 (CX30); gap junction protein that forms transmembranous gap junction channels that connect adjacent cells	Brain; skin
0.043	1.44	0.43	0.33	0.56	1.40	0.38	ET63410	Semaphorin Hv; a novel member of semaphorin gene family; secreted glycoprotein involved in embryonic development	Developing lungs ; skeletal elements; neural tubes
<0.001	1.12	0.04	0.09	0.15	1.12	0.21	X53257	Neurotrophin-3 (NT-3); secreted protein; binds high affinity receptor trk C	Liver parenchyma) cells olfactory bulb cerebellum; septum and hippocampus; thymus, heart, diaphragm, pancreas, spleen, kidney, adrenal gland
0.004	1.16	0.28	0.22	0.26	1.22	0.19	ET61665	Discs-large tumor suppressor homologue (dlgh1); important role in the localization and function of glutamate receptors and K(+) channels; member of the MAGUK (membrane associated guanylate kinase homologue) family of proteins	Localized to synapse; epithelial cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	1.05	0.20	0.16	0.27	1.32	0.15	ET63395	Axonemal dynein heavy chain (mdhc1); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit motor ATPase complexes called dyneins	Brain, trachea, testis
0	1.21	0.17	0.00	0.00	0.95	0.09	ET63399	Axonemal dynein heavy chain (mdhc3); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit motor ATPase complexes called dyneins	Brain, trachea, testis
0.013	1.09	0.08	0.21	0.36	1.30	0.42	ET63402	Axonemal dynein heavy chain (mdhc6); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit motor ATPase complexes called dyneins	Brain, trachea, testis
0.002	1.07	0.09	0.24	0.21	1.14	0.23	ET63405	Axonemal dynein heavy chain (mdhc9); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit motor ATPase complexes called dyneins	Brain, trachea, testis

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0	1.02	0.08	0.05	0.09	1.14	0.08	Y08485	Synaptonemal complex protein 3; part of the lateral element of the synaptonemal complex; a meiosis-specific protein structure essential for synapsis of homologous chromosomes	Testis; synaptonemal complex protein 1 is also expressed in embryonic ovary, adult brain and testis
0.007	1.20	0.53	0.00	0.00	1.28	0.30	L28756	Gonadotropin-releasing hormone receptor; G-protein-coupled receptor; GnRH activates all four MAPK cascades by a PKC-dependent mechanism.	Anterior pituitary, brain and reproductive organs as well as many steroid-dependent tumor tissues
0.003	1.18	0.24	0.29	0.23	1.09	0.10	Z46845	Preproglucagon; glucagon-like peptide I and II; member of vasoactive intestinal peptide (VIP)/secretin/glucagon/GHRH family of neuropeptides	Pancreatic alpha cells, ileum + K41, CNS
<0.001	1.09	0.15	0.08	0.11	1.10	0.15	U66201	Fibroblast growth factor homologous factor 1 (FGF-1); nervous system development and function	Highest expression in brain and skeletal muscle
<0.001	1.13	0.15	0.00	0.00	0.99	0.18	Z27088	Relaxin precursor (rlx); relaxin; member of insulin gene family; remodeling of collagen and uterine contractility	Brain, uterus, prostate gland, pancreas and kidney, with other tissues giving weak signals

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.005	1.20	0.24	0.25	0.22	1.23	0.29	ET62740	Ankyrin-3 (Ank3); also called ankyrin(G); skeletal protein implicated in Na(+) channel clustering; essential for clustering NaCh and neurofascin at axon initial segments and is required for physiological levels of sodium channel activity	Widely distributed, especially in epithelial tissues, muscle, and neuronal axons
0.007	1.37	0.08	0.34	0.35	1.05	0.25	U48397	Mercurial-insensitive water channel 1 (mMIWC1); allows water and small solutes to pass	Brain, eye, lung, kidney, heart, muscle
0.004	1.16	0.28	0.22	0.26	1.22	0.19	ET61665	Discs-large tumor suppressor homologue (dlgh1) gene; important role in the localization and function of glutamate receptors and K(+) channels; member of the MAGUK (membrane associated guanylate kinase homologue ues) family of proteins	Localized to synapse; epithelial cells
0.005	1.26	0.03	0.15	0.26	1.16	0.39	M30440	Potassium channel gene (MK2); shaker subfamily	T cells; myelinating Schwann cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0	1.00	0.15	0.02	0.03	1.15	0.10	U58367	Neuropeptide Y receptor Y5/Y6/Y2b (referred to as both Y5 and Y2b, has now been designated as Y6 in literature); (NPY-Y6); neuropeptide Y is an important regulator of energy balance in mammals through its orexigenic, antithermogenic, and insulin secretagogue actions; expressed abundantly in the central nervous system; NPY receptors mediate a variety of physiological responses including feeding and vasoconstriction	Neurons, vascular smooth muscle cells
0.000	1.06	0.15	0.00	0.00	1.56	0.37	ET61090	Ryanodine receptor type-3; intracellular Ca2+ channels	Skeletal and smooth muscle, CNS
0.006	0.96	0.24	0.18	0.32	1.10	0.10	ET62978	Neosin/lark; RNA-binding protein; Drosophila homologue encodes an element of the clock output pathway regulating adult eclosion (circadian rhythm)	Uncharacterized, probably neuronal

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	1.24	0.31	0.00	0.00	1.18	0.27	D50095	Histamine H1 receptor; GTP-binding protein-coupled receptor; coupled to phosphoinositide turnover-calcium mobilization signaling pathway; regulates insulin-like growth factor I expression and cell proliferation; modulates IL-6 action; regulates physiological functions in neurons; regulates transport of thyroxine into hepatocytes	Liver, brain, spleen (ubiquitous)
0	1.09	0.16	0.00	0.00	1.15	0.13	U60330	Ki antigen (PA28 gamma); cell proliferation; the interferon-gamma (IFN-gamma)-inducible PA28 activator complex enhances the generation of class I binding peptides by altering the cleavage pattern of the proteosome	Liver, neurons, broad tissue distribution
0	1.09	0.16	0.00	0.00	1.18	0.18	Z31663	Activin type IB receptor; limb development; expressed coincidently with the formation of the last phalanx of each digit	Embryo: brain, some ganglia, vibrissae, lungs, body wall, stomach, gonads, ribs, limbs, shoulders, olfactory region, eye, tooth primordium, esophagus, mesonephros, dorsal root ganglia and is strongly expressed in the spinal cord.

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	1.02	0.35	0.00	0.00	1.31	0.16	U33535	Fibroblast growth factor 9 (FGF-9); autocrine and paracrine growth factor; prevents cell death in cultured motoneurons; plays a role in embryonic neural cell differentiation; thrombopoietic activity (acts on the in vivo proliferation of megakaryocytes)	Adult and developing CNS: neurons, astrocytes, oligodendrocytes, glial cells, epithelial cells, brain, kidney, prostate (stromal cells); in embryogenesis expressed in many areas including intermediate mesoderm
NOT REPORT IN LIVER MUSCLE									
0.008	1.27	0.33	0.27	0.27	1.04	0.13	X04405	Myoglobin gene; small globular heme protein; binds gaseous ligands such as O2, CO and NO	Muscle
0.002	1.32	0.20	0.00	0.00	0.80	0.39	X92523	Skeletal muscle-specific calpain (canp3); intracellular calcium-dependant cysteine proteinase; tissue specific myofibrogenesis, modifies ryanodine receptor Ca2+ release channel	Skeletal muscle; differentially spliced variants in smooth muscles during fetal period
0.001	1.14	0.15	0.15	0.27	1.17	0.15	M92416	Fibroblast growth factor (Fgf6); Fgf6 is the only known member of the FGF family whose expression is restricted to the muscle cell lineage during development	Skeletal muscle
0.012	1.33	0.35	0.00	0.00	1.45	0.67	M14537	Nicotinic acetylcholine receptor beta subunit	Skeletal muscle
0.003	1.09	0.12	0.14	0.24	1.11	0.31	X55718	Nicotinic acetylcholine receptor e subunit; embryonic	Skeletal muscle

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.003	1.08	0.04	0.00	0.00	1.19	0.47	Z67747	ZT3 zinc finger factor	Skeletal, cardiac muscle, and spleen in adult
0.01	1.15	0.12	0.02	0.04	1.29	0.62	U37353	Protein phosphatase 2A regulatory subunit	Skeletal and heart muscle
0.005	0.93	0.18	0.00	0.00	1.14	0.14	ET62103	Nebulin; a family of giant myofibrillar proteins	
0.002	1.18	0.18	0.24	0.21	1.14	0.22	ET62883	Skeletal muscle chloride channel	Skeletal muscle
0.023	1.45	0.58	0.00	0.00	1.16	0.61	ET63019	Skeletal muscle ryanodine receptor; calcium release channel	Skeletal muscle
0	1.02	0.07	0.00	0.00	1.27	0.23	ET62998	Dystrobrevin:postsynaptic protein; important in the formation and maintenance of the mammalian neuromuscular junctions.	Skeletal muscle
0.001	1.19	0.25	0.25	0.05	1.21	0.20	ET62865	Alpha 4 integrin; a leukocyte glycoprotein involved in both cell-extracellular matrix and cell-cell interaction	Skeletal muscle
0	0.98	0.21	0.00	0.00	1.25	0.05	U49393	Sarcoendoplasmic reticulum Ca2+ ATPase; ion pump	Skeletal, smooth, and cardiac muscle
0.005	1.20	0.24	0.25	0.22	1.23	0.29	ET62740	Ankyrin-3 (Ank3); also called ankyrin(G); skeletal muscle protein implicated in Na(+) channel clustering; essential for clustering NaCh and neurofascin at axon initial segments and is required for physiological levels of sodium channel activity.	Widely distributed, especially in epithelial tissues, muscle, and neuronal axons

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.007	1.37	0.08	0.34	0.35	1.05	0.25	048397	Mercurial-insensitive water channel 1 (mMIWC1); allows water and small solutes to pass	Brain, eye, lung, kidney, heart, muscle
0.000	1.06	0.15	0.00	0.00	1.56	0.37	ET61090	Ryanodine receptor type-3; intracellular Ca2+ channels	Skeletal and smooth muscle, CNS
0.005	1.17	0.23	0.43	0.16	1.24	0.23	X83932	Ryanodine receptor type 1 (RYR1 gene); intracellular calcium channel	Skeletal muscle
0.004	1.25	0.48	0.00	0.00	1.28	0.24	X80417	MB-IRK2 (second class of inward rectifier potassium channels); ion channel	Heart, kidney, and skeletal muscle
VASCULAR SMOOTH MUSCLE									
0	1.00	0.15	0.02	0.03	1.15	0.10	U58367	Neuropeptide Y receptor Y5/Y6/Y2b (referred to as both Y5 and Y2b, has now been designated as Y6 in literature); (NPY-Y6); neuropeptide Y is an important regulator of energy balance in mammals through its orexigenic, antithermogenic, and insulin secretagogue actions; expressed abundantly in the central nervous system; NPY receptors mediate a variety of physiological responses including feeding and vasoconstriction	Neurons, vascular smooth muscle cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.021	1.40	0.24	0.00	0.00	0.79	0.71	J03293	Phosphorylase kinase, gamma subunit; phosphorylates and activates glycogen phosphorylase, the enzyme that initiates the catabolism of glycogen in skeletal muscle	Heart, skeletal and cardiac muscle (not in liver and the liver gamma subunit does not cross-hybridize with the skeletal muscle gamma subunit cDNA)
0.044	1.12	0.26	0.61	0.27	1.32	0.28	U36757	Thrombin receptor (PAR-1); Transmembrane G-protein coupled receptor; activated by serine protease cleavage; thrombin is a serine protease generated by the activation of the blood coagulation cascade following vessel injury; thrombin acts as a mitogen and apoptosis inducer	Blood, platelets, monocytes; endothelial cells; cardiomyocytes; neuronal and glial cells
0.000	1.06	0.15	0.00	0.00	1.56	0.37	ET61090	Ryanodine receptor type-3; intracellular Ca^{2+} channels	Skeletal and smooth muscle, CNS
NOT REPORTED IN LIVER; SIGNAL TRANSDUCTION									
0.013	0.77	0.51	0.00	0.00	1.10	0.15	L12460	Mouse cyclic GMP-dependent protein kinase II; signal transduction	Brain, kidney, small intestine, colon
0.000	0.96	0.15	0.00	0.00	1.48	0.12	Y00850	Mouse Kruppel-like gene (MKr2); differentiation and/or phenotypic maintenance of neurons	Restricted to central and peripheral neurons of adults
0.022	1.13	0.08	0.40	0.24	1.04	0.35	Z23143	Activin receptor-like kinase-6; receptors for morphogenic proteins; serine-threonine kinase	Developing mesenchyme, muscle, blood vessels, CNS, ear, eye, epithelium

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.044	1.26	0.38	0.44	0.39	1.12	0.12	X66983	Rck gene; protein kinase	Photoreceptors, olfactory receptors, respiratory and choroid plexus epithelial cells, germ cells
0.048	1.79	1.06	0.00	0.00	1.18	0.54	U33005	Tbc1; domains homologous to tre-2 oncogene and yeast mitosis regulators BUB2 and cdc16; nuclear localization	Not well-characterized; hematopoietic cells, testis and kidney
<0.001	0.98	0.18	0.03	0.03	1.08	0.11	Y12293	LUN transcription factor; forkhead domain identical to the HFH8 gene; C-terminal region similar to the HFH8 gene	Lung bronchiolar epithelium and type II pneumocytes; tissue distribution not well characterized
<0.001	1.18	0.20	0.00	0.00	1.04	0.15	U48721	Zinc finger protein 60 (ZFP60); Kruppel associated boxes	Expressed transiently during in vitro muscle differentiation
0.003	1.43	0.41	0.07	0.12	1.33	0.34	D13801	DNA-binding transcription factor (Emb); class VI POU domain	CNS; tissue distribution not well characterized
0.026	1.07	0.69	0.00	0.00	1.06	0.09	X63963	Paired box protein (Pax-6); transcription factor	Development of eye and CNS
<0.001	1.07	0.02	0.00	0.00	1.07	0.19	U76208	Neurogenin 3 (ngn3); transcription factor; neuroD-related bHLHprotein	CNS and early precursors of pancreatic endocrine cells (embryogenesis)
0.003	1.15	0.31	0.07	0.12	1.28	0.32	U17252	Metabotropic glutamate receptor 8; G-protein coupled	CNS, glial cells; retina and olfactory bulb; stellate/basket cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
<0.001	1.02	0.02	0.04	0.06	1.06	0.17	U14420	GABA-benzodiazepine receptor beta-3 subunit; link binding of GABA (gamma-aminobutyric acid) to inhibitory chloride flux	CNS
<0.001	1.11	0.22	0.09	0.16	1.07	0.07	X66118	Glutamate receptor subunit GluR5-2c.	Brain
0.001	1.11	0.33	0.06	0.10	1.05	0.04	Z14224	5HT1E beta serotonin receptor; G protein-coupled receptor	Brain; tissue distribution not well characterized
0.001	1.21	0.29	0.02	0.03	1.26	0.29	X79082	MDK1 (mouse developmental kinase 1); member of receptor tyrosine kinase family	Brain, testes and spleen
<0.001	1.20	0.22	0.01	0.01	1.30	0.27	Z48757	Intestinal tyrosine kinase; protein tyrosine kinase	Mammary gland and intestine
0.001	1.11	0.16	0.00	0.00	1.19	0.37	X58287	MR-PTPmu; receptor-like protein tyrosine phosphatase	Lung, brain, heart
<0.001	1.11	0.17	0.00	0.00	1.14	0.14	M61000	Bombesin/gastrin-releasing peptide receptor; member of the G protein-coupled receptor family	Fibroblasts
0.001	1.20	0.18	0.09	0.16	1.00	0.25	ET61461	G-protein coupled receptor; poorly characterized	Unknown
0.009	1.14	0.10	0.50	0.18	0.96	0.20	ET63226	Nude gene (Whn) winged helix transcription factor family; modulates growth factor production by differentiating epithelial cells including keratinocytes; also controls development of the immune system in thymus.	In adult thymus and skin; embryonic nails, nasal passages, tongue, palate and teeth

P	CR	std	CON	std	SW	std	GenBank	Description	Location
NOT REPORTED IN LIVER, HORMONE / GROWTH FACTOR									
0.007	1.09	0.08	0.39	0.27	1.11	0.20	M22740	Thyrotropin beta-subunit (TSH-beta)	Pituitary
0.007	1.00	0.11	0.39	0.27	1.11	0.12	U12932	Follicle stimulating hormone beta subunit (FSH-beta, gonadotropin); stimulates development of follicle and spermatogenesis	Gonadotropes of the anterior pituitary
0.004	0.91	0.33	0.21	0.34	1.46	0.09	U25145	Lutenizing hormone beta subunit; regulation of reproduction	Gonadotropes of the anterior pituitary
NOT REPORTED IN LIVER; EMBRYONIC DIFFERENTIATION TRANSCRIPTION FACTORS									
0.001	1.06	0.09	0.00	0.00	0.98	0.33	X51683	T gene (Brachyury gene); T-box family; sequence specific DNA-binding protein that functions as a transcription activator; required for morphogenesis of mesoderm-derived structures, control of gastrulation; development of the heart; perhaps limb formation	Early embryogenesis; mesoderm formation; heart and limb morphogenesis
0.001	1.09	0.10	0.00	0.00	1.17	0.37	Z15103	Mox-1; homeobox gene; marker of epithelial-mesenchymal transformation	Early embryogenesis; mesodermal patterning in mouse embryos
0.001	1.08	0.08	0.22	0.18	0.91	0.21	X86368	Fkh-2; a forkhead/winged helix transcription factor	Numerous tissues of embryo, including paraxial mesoderm, somites, branchial arches, vibrissae, central nervous system and kidney

P	CR	std	CON	std	SW	std	GenBank	Description	Location
<0.001	1.10	0.02	0.00	0.00	1.09	0.19	X97817	Semaphorin F; involved in axonal guidance	Early embryogenesis
0.018	1.78	0.50	0.40	0.46	1.10	0.21	S80989	NK-related homeobox gene (Nkx-5.2); cell type specification of neuronal cells	Developing CNS and ear in E13.5 embryos; cell type specification of neuronal cells
0.011	1.00	0.21	0.50	0.24	1.22	0.13	X83577	K-glypican; cell surface heparin sulfate proteoglycan; suggested role in regulating cell cycle progression during the transition of neural cells from proliferation to differentiation.	In embryo major sites are tubular epithelial cells in kidney and proliferating neuroepithelial cells in brain; neurons
0	1.03	0.03	0.11	0.10	1.08	0.21	M34094	Retinoic acid-responsive protein (MK); growth differentiation factor	Mid-gestation mouse embryogenesis; not reported in normal adult liver
0	1.11	0.09	0.00	0.00	1.03	0.15	M93128	Homeobox transcription factor (EVX2); limb development	Embryo
0	1.04	0.15	0.13	0.12	1.16	0.10	X16510	Hox 3.3; homeobox transcription factor; embryogenesis; haematopoiesis	Spleen, bone marrow
0	1.09	0.16	0.00	0.00	1.18	0.18	Z31663	Activin type IB receptor; limb development; expressed coincidently with the formation of the last phalanx of each digit	Embryo: brain, some ganglia, vibrissae, lungs, body wall, stomach, gonads, ribs, limbs, shoulders, olfactory region, eye, tooth primordium, esophagus, mesonephros, dorsal root ganglia and is strongly expressed in the spinal cord.

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0	0.99	0.06	0.12	0.20	1.10	0.13	D78175	Atria natriuretic peptide clearance receptor (ANP-CR or NPRC); membrane protein; modulates availability of natriuretic peptides at target organs; activation of G protein-coupled signaling system; endothelial permability; may modulate angiogenesis by inhibition of vascular endothelial cell growth factor; modulates activity of mitogen-activated protein kinase (MAPK, regulation of cell proliferation)	Epithelial and endothelial cells; lung (smooth muscle cells), heart (aortic smooth muscle cells), placenta
NOT REPORTED IN LIVER; Other									
0.000	1.43	0.07	0.18	0.21	1.05	0.22	Z38118	Synaptonemal complex protein 1 (SCP1); pairing of chromosomes during meiosis	Testis
0.003	0.86	0.29	0.11	0.19	1.21	0.19	U61085	Thiazide-sensitive sodium and chloride cotransporter; transmembrane protein	Kidney
0.024	1.20	0.36	0.34	0.30	1.46	0.44	X95226	Dystrobrevin; formation and maintenance of mammalian neuromuscular junction	CNS
0.000	1.38	0.32	0.00	0.00	0.96	0.10	U73915	Membrane metalloendopeptidase homologue (Pex); mineralization of extracellular matrix by osteoclasts	Kidney, bone
0.011	1.10	0.10	0.35	0.38	1.13	0.13	M10114	Kappa-casein; milk protein	Mammary glands

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.037	1.32	0.29	0.14	0.24	0.90	0.63	X99251	Repetin; similar to intermediate filament-associated proteins profilaggrin and trichohyalin; expression during late epidermal differentiation	Epithelia of forestomach and tongue
0.006	1.26	0.18	0.30	0.22	0.98	0.29	U66204	Fibroblast growth factor homologous factor 4 (FHF-4); involved in nervous system development and function	CNS
0.004	1.06	0.10	0.36	0.27	1.10	0.10	M36516	Zinc finger proteins (mkr3,4,5)	Whole embryo, testes in adult
0.034	0.96	0.30	0.32	0.40	1.14	0.12	X86000	N-glycan alpha 2,8-sialyltransferase (STSia IV)	Lung, heart, spleen, brain
0.025	1.21	0.11	0.40	0.36	1.12	0.33	M25513	Rod transducin alpha subunit (Tr-alpha); couples photolysis of rhodopsin to activation of cGMP phosphodiesterase; visual signal casade	Retina, not reported in liver, kidney, heart
0.008	1.18	0.51	0.23	0.40	1.82	0.25	X12875	Neural cell adhesion molecule L1 (N-CAM L1); involved in Ca2 + independent neural adhesion	Nerve cells
0.049	1.44	0.41	0.49	0.38	1.12	0.30	Y00500	Glandular kallikrein mGK-5; serine protease	Salivary glands; possible crosshybridization with liver kallikreins
0.030	1.18	0.07	0.29	0.45	0.85	0.25	X63100	Connexin45; gap junction protein; ion exchange channels	Lung, brain,, heart, intestine; embryonic brain, skin, and kidney

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.036	1.30	0.18	0.41	0.45	1.06	0.28	ET62673	Hyaluronan synthase 3; polymerizes hyaluron, (extracellular) glycosaminoglycan; can be hallmark of tissue remodeling; reduces cell motility; hyaluron found throughout the extracellular matrix, especially in soft connective tissue	Eyes, kidney, chondrocytes
0.017	1.81	0.92	0.00	0.00	1.12	0.11	D87471	Actin capping protein; germ cell gene 3 (gsg3); homologue of somatic cell type actin capping protein alpha (ACP alpha)	Haploid germ cells of testis
0.019	1.16	0.14	0.48	0.30	1.26	0.29	J04847	PL10; ATP-dependent RNA helicase; suggested role in spermatogenesis; protein homologous to eIF-4A	Testis (not reported in liver)
<0.001	1.09	0.14	0.01	0.02	1.19	0.29	Z46299	Sp17 gene for sperm specific protein; calmodulin binding protein	Mammalian testis; sperm-specific
<0.001	1.05	0.05	0.14	0.23	1.08	0.09	X72697	Meiosis-specific XMR; transcriptional activator function?	Testis; lymphoid cell lineages; nuclei of spermatocytes, early in the prophase of the first meiotic division, and later becomes concentrated in the XY nuclear subregion
<0.001	1.20	0.18	0.02	0.02	1.07	0.26	M19413	Testicular alpha tubulin	Testis
<0.001	1.17	0.20	0.08	0.14	1.01	0.01	X96606	Ott, mouse X-linked multigene family	Expressed during meiosis

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.002	1.14	0.31	0.13	0.22	1.23	0.10	D13664	Osteoblast specific factor 2 (OSF-2); extracellular matrix?	Osteoblastic cells
0.006	1.33	0.43	0.09	0.15	1.40	0.39	X15830	Neuroendocrine protein 7B2; secretory protein present in serum; proteolytic conversion and activation of proprotein convertases 2 in the endoplasmic reticulum	Widely distributed neuroendocrine protein; neurons, endocrine cells; pituitary, cells producing insulin and glucagon; melanosomes
0.440	1.10	0.80	0.53	0.59	1.27	0.67	D38162	Alpha1(XI) collagen (COL11a1); structural integrity; essential for normal cartilage development	Embryo cartilaginous tissue, brain, heart, tongue, intestine, and otic vesicles
0.002	1.01	0.03	0.43	0.37	1.56	0.04	M35732	Seminal vesicle secretory protein IV (SVS IV); major secretory protein of seminal vesicles; regulation of the immune response, blood coagulation; inflammatory reaction; reproduction	Seminal vesicles
0.043	1.87	0.72	0.00	0.00	0.97	0.95	X05260	Y chromosome RNA transcript expressed in testis (pY353/B); protein product uncharacterized; sex determination factor?	Testis (reported not present in adult liver)
0.046	2.86	0.65	0.00	0.00	1.46	1.74	U96701	Intracellular serine proteinase inhibitor (mBM2A); serine proteinase inhibitors (serpins) are regulators of extracellular proteolysis	Predominantly in testis

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.019	1.42	0.39	0.58	0.06	1.11	0.20	ET63122	Beta-Tectorin; extracellular matrix protein	Inner ear; expressed by cells in and surrounding the mechanosensory epithelia in embryo and adult
0.005	1.00	0.05	0.25	0.31	1.15	0.21	X04724	Preproinsulin gene II	Pancreas and islets
0.004	1.21	0.39	0.06	0.11	1.63	0.46	X04725	Preproinsulin gene I	Pancreas and islets
0.002	1.51	0.44	0.00	0.00	1.20	0.32	ET63205	Odorant binding protein 1b	Nasal epithelium.
0	1.18	0.33	0.00	0.00	1.51	0.21	ET63408	Capping protein beta 3 subunit; a novel isoform of actin-binding protein; a component of the cytoskeletal calyx of the mammalian sperm head.	Spermiogenesis
0.045	1.24	0.21	0.00	0.00	0.93	0.81	X58169	T-complex responder (Tcp-10); Tcp-10 gene has been established as a molecular candidate for the T complex responder locus which plays a central role in the. transmission ratio distortion phenotype expressed by males heterozygous for a T haplotype.	Male germ line
0.011	1.45	0.81	0.00	0.00	1.72	0.25	ET61364	Meprin beta subunit isoform (Mep-1beta); meprins are membrane-bound oligomeric metalloendopeptidases, contain alpha and/or beta subunit	Kidney, intestine, not reported in liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.008	1.19	0.16	0.22	0.19	1.37	0.47	ET62832	Perforatorial protein (PERF 15); a novel testicular protein; sequence similarities to a family of lipid binding proteins; major component of the rat sperm perinuclear theca.	Testis
0.016	1.29	0.33	0.24	0.26	1.17	0.40	ET62968	Odorant receptor 23 (OR23)	Olfactory and testicular cells
0.003	0.94	0.45	0.02	0.04	1.21	0.02	ET63528	A-myb; a conserved member of the Myb proto-oncogene family, encodes a sequence-specific DNA binding protein (A-Myb) that binds to and transactivates promoters containing myb- binding sites	Abundant expression in testis (germ cell differentiation); low level expression in ovary, spleen (B lymphocytes) and brain; CNS in embryos
0.005	1.44	0.46	0.00	0.00	1.14	0.39	ET63177	Pax-4; a paired-box transcription factor that plays an important role in the development of pancreatic beta/delta cells; role in endocrine cell development	Pancreatic islet endocrine progenitor cells
0.001	1.06	0.22	0.04	0.07	1.37	0.33	M20567	Heat-shock-like protein 70-2 (HSP70.2); not induced by heat shock; developmentally regulated in spermatogenic , cells; critical role in spermatogenesis	Male germ cells
0.001	1.30	0.05	0.00	0.00	1.10	0.38	ET61399	G protein alpha olfactory subunit; sensory transduction	Olfactory epithelium
0.015	1.07	0.08	0.09	0.15	1.16	0.57	L28819	Involucrin; a glycine-,serine- and cysteine-rich protein expressed late in differentiation of grandular layers in normal epidermis	Epidermis

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.005	1.45	0.52	0.05	0.09	1.17	0.26	ET62336	DNA ligase III-beta; DNA ligase III exists as two distinct isoforms denoted alpha and beta	Alpha is expressed in most tissues; beta is expressed in testes and during spermatogenesis
0	0.95	0.18	0.07	0.12'	1.13	0.11	D49438	25-hydroxyvitamin D3 24-hydroxylase; metabolism and regulation of vitamin D3	Kidney and intestine.
0.011	1.27	0.28	0.00	0.00	1.22	0.59	M26940	Beta-casein gene	Mammary glands
0.001	1.03	0.29	0.05	0.05	1.12	0.10	V00740	Epsilon-casein	Mammary glands
0	1.01	0.08	0.02	0.04	1.07	0.07	V00428	Lysozyme; signaling molecule for mast cells which respond with histamine secretion	Macrophages, paneth cells (located in duodenal crypts)
BLOOD, NOT IgG									
0.037	1.24	0.26	0.45	0.42	1.09	0.16	U86405	Amphiphysin II; endocytosis and signal transduction (recycling synaptic vesicle components)	Macrophages, neurons, germ cells, endocrine tissues
0.013	1.02	0.02	0.11	0.19	0.94	0.44	U69136	T1-cadherin, calcium-binding membrane glycoprotein acting as cell adhesion molecule (CAMs).	Thymocytes
0.006	1.31	0.40	0.16	0.20	1.32	0.30	X53176	Integrin alpha-4; cell adhesion	Lymphocytes
<0.001	1.27	0.15	0.03	0.05	1.14	0.24	X91043	Erythrocyte band 7 integral membrane protein; protein 7.2b; stomatin	Spleen, lung, testis, not reported in liver
<0.001	1.06	0.06	0.14	0.12	1.12	0.15	X15592	CTLA-2-beta; homologue to cysteine protease proregion	T cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.002	1.11	0.15	0.15	0.26	1.12	0.20	X97227	Cell surface glycoprotein CD53; pan-leukocyte antigen; cell membrane glycoprotein	Thymocytes
<0.001	1.10	0.20	0.03	0.05	1.27	0.19	U43384	Gp91phox (Cybb); phagocyte cytochrome b558; heterodimer comprised of gp91 phox and p22phox; a flavocytochrome that mediates the transfer of electrons from NADPH to molecular oxygen in the respiratory burst oxidase	Phagocyte
0.005	1.26	0.03	0.15	0.26	1.16	0.39	M30440	Potassium channel gene (MK2); shaker subfamily	T cells; myelinating Schwann cells
0.002	0.99	0.29	0.00	0.00	1.35	0.35	X52991	Homologue of the rat T cell differentiation marker RT6; cell-cell signaling	Cytotoxic T lymphocytes
0.000	1.37	0.11	0.00	0.00	0.97	0.11	X14092	MCSP-1 CTL serine protease 1; may play a role in cytolytic lymphocyte activation	T lymphocytes
0.033	1.36	0.33	0.29	0.50	1.07	0.30	U04269	Interleukin-1 beta converting enzyme (ICE); may mediate endotoxin-induced cholestatic effect of decreased hepatocellular bile salt uptake; IL-1 DOWN in CR	Monocytes and macrophages
0.015	1.47	0.47	0.24	0.41	1.07	0.06	L16928	Differentiation antigen (CD22); mediates B cell interactions with endothelial cells	B cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.027	1.20	0.13	0.24	0.34	0.94	0.44	ET62844	Immunoglobulin-like receptor PIRA6 (12M1); appears to activate immunoglobulin-related receptor	B lymphocytes, myeloid lineage cells
0.046	1.31	0.39	0.31	0.50	0.98	0.18	U49866	Class I recognizing receptors involved in ability of F1 hybrid mice to reject parental H-2d bone marrow cell grafts	Subpopulation of natural killer cell
0.003	1.07	0.07	0.38	0.21	1.05	0.17	X04123	Terminal deoxynucleotidyltransferase; template-independent DNA polymerase; VDJ assembly; recombination	Earliest stage B and T cells
0.007	1.53	0.51	0.00	0.00	1.51	0.54	U25691	Lymphocyte specific helicase; putative role in replication, repair, recombination and transcription	T and B cells at both the immature and mature stage; not in heart, liver, lung, muscle, brain or kidney
0.005	1.07	0.45	0.00	0.00	1.93	0.62	M23501	P500/TCA3; SIS-epsilon; small, secreted, and inducible protein; expressed more abundantly in activated mouse helper T cells than by resting T cells	T cells, myeloid and lymphoid cells
0.007	1.08	0.13	0.20	0.34	1.11	0.23	ET61471	Mast cell protease 7 (mMCP-7); mouse mast cell tryptase 2; released when .mast cells are activated	Mast cells
0.001	1.10	0.31	0.00	0.00	1.41	0.33	M55617	Mast cell protease-4	Peritoneal and most connective tissue '

P	CR	std	CON	std	SW	std	GenBank	Description	Location
BLOOD HORMONE / CYTOKINE / CHEMOKINE / SIGNAL TRANSDUCTION / RECEPTOR									
0.038	1.06	0.10	0.30	0.53	1.15	0.21	X51468	Preprosomatostatin; precurser peptide cleaved to release somatostatin which regulates T cell IFN-gamma production	Macrophages; nervous system
0.044	1.53	0.46	0.17	0.30	1.03	0.69	M16762	Interleukin 2 (IL-2); stimulates proliferation of activated T lymphocytes	Helper T cells
0.014	1.06	0.06	0.54	0.18	1.30	0.34	X54542	Interleukin-6; promotes B cell maturation to Ig- secreting cells; helps activate T cells	Some helper T cells and macrophages
0.004	1.00	0.08	0.22	0.38	1.40	0.24	X07962	Interleukin 7 (IL-7); growth factor	B cell progenitors
0.000	1.52	0.10	0.00	0.00	0.97	0.19	U28404	Macrophage inflammatory protein-1 alpha receptor; mediates growth inhibitory effects of the chemokine	MIP-1 alpha RL2 in liver and spleen
0.001	1.38	0.28	0.12	0.21	1.00	0.17	X53798	Macrophage inflammatory protein-2 (MIP2); small inducible cytokine subfamily member	Macrophages
<0.001	0.99	0.11	0.07	0.13	1.25	0.22	ET62976	Macrophage inflammatory protein receptor 1-alpha 2; Induces mobilization of intercellular calcium; beta-chemokine; leucocyte chemoattractant	Thymus, heart, spleen, and liver; to lesser extent in the lung and brain

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.015	1.31	0.23	0.44	0.35	1.04	0.13	V00755	Interferon beta (type 1); growth factor; T helper cell differentiation factor; antiviral; modulates immune responses to foreign and self-antigens	Ubiquitous
<0.001	1.21	0.19	0.11	0.19	1.10	0.11	V00756	Interferon beta (type 2)	T cells
0.015	1.31	0.52	0.00	0.00	1.19	0.50	M26271	Interleukin 2 receptor; cytokine receptor	T cells
0.016	1.47	0.65	0.14	0.21	1.08	0.10	M35684	Complement receptor type 2 (CR2)	Late pre-B cells
0.010	1.22	0.69	0.05	0.09	2.15	0.64	L41495	Protein-serine/threonine kinase (pim-2); cell proliferation; highly expressed in mitogenically (cytokines) stimulated hematopoietic cells; evokes long-term potentiation in hippocampus	Blood, epithelial and CNS embryonic development '
0.002	2.00	0.36	0.00	0.00	1.17	0.55	ET61263	Cytosolic tyrosine protein kinase SYK; signal transduction	Lymphopoiesis; haematopoietic cells, platelets, macrophages and neutrophils
<0.001	1.07	0.11	0.02	0.03'	1:11	0.10	X58995	Calmodulin-dependent protein kinase IV; multifunctional, serine-threonine protein kinase	T cells
0.002	1.22	0.42	0.00	0.00	1.14	0.13	D30743	Wee1 kinase; inhibits entry into mitosis by phosphorylation of the Cdc2 kinase	Lymphocytes

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.044	1.12	0.26	0.61	0.27	1.32	0.28	U36757	Thrombin receptor (PAR-1); transmembrane G-protein coupled receptor; activated by serine protease cleavage; thrombin is a serine protease generated by the activation of the blood coagulation cascade following vessel injury; thrombin acts as a mitogen and apoptosis inducer.	Blood, platelets, monocytes; endothelial cells; cardiomyocytes; neuronal and glial cells
<0.001	1.15	0.13	0.00	0.00	1.05	0.17	U36575	T cell transcription factor NFAT1 isoform B	T cells
<0.001	1.02	0.16	0.00	0.00	1.13	0.15	Z11664	Son of sevenless 2; Ras-specific exchange factors	T cells
0.002	1.18	0.17	0.16	0.27	1.22	0.25	Z11574	Son of sevenless 1; Ras-specific exchange factors	T cells
0.026	0.82	0.47	0.19	0.34	1.43	0.39	M36654	Homeobox gene 2.6 (Hox-2.6) transcription factor; embryonic development; haematopoiesis	Whole embryo; in adult: blood cells, stem cells and low levels in somatic and spermatogenic cells
0.034	1.30	0.84	0.00	0.00	1.22	0.24	U10092	Ly-49F-GE antigen; NK cell suface molecule; determinant of IL-2-activated NK cell specificity; inhibitory receptor for interaction with MHC class I	NK cells
0.003	1.15	0.23	0.27	0.12	1.17	0.24	L43567	Antigen, B cell receptor	Blood

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.007	1.08	0.13	0.20	0.34	1.11	0.23	ET61471	Mast cell protease 7 (mMCP-7), mast cell tryptase 2; released when mast cells are activated	Mast cells
0	1.14	0.05	0.00	0.00	0.96	0.26	ET61424	Protein-tyrosine phosphatase epsilon precursor; the protein tyrosine phosphatase epsilon (PTPepsilon) gene gives rise to two proteins: a transmembranal, receptor-like form and a cytoplasmic, non-receptor form	Hematopoietic tissues
0.003	1.31	0.57	0.00	0.00	1.63	0.18	ET62920	CC Chemokine Receptor-4; integral membrane protein; G- protein coupled receptor; signals involve chemotaxis and calcium flux; directs cell movement in thymus; directs monocytes and lymphocytes to their target tissues	Thymus, T cells, and monocytes
0	1.04	0.15	0.13	0.12	1.16	0.10	X16510	Hox 3.3; homeobox transcription factor; embryogenesis; haematopoiesis	Spleen, bone marrow
0.044	1.12	0.26	0.61	0.27	1.32	0.28	U36757	Thrombin receptor (PAR-1); transmembrane G-protein coupled receptor; activated by serine protease cleavage; thrombin is a serine protease generated by the activation of the blood coagulation cascade following vessel injury; Thrombin acts as a mitogen and apoptosis inducer.	Blood, platelets, monocytes; endothelial cells; cardiomyocytes; neuronal and glial cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.000	1.31	0.06	0.00	0.00	0.98	0.06	ET62839	Immunoglobulin-like receptor PIRA1; activating receptor on murine B lymphocytes, dendritic cells, and myeloid-linage cells	B lymphocytes; dendritic cells, myeloid-linage cells.
BLOOD TRANSCRIPTION FACTOR									
0.029	1.07	0.25	0.18	0.31	1.77	0.83	X06762	Hox2.3; homeobox gene; embryo development; haematopoiesis	Whole developing embryo; blood; bone marrow cells; natural killer cells
0.012	1.10	0.20	0.46	0.27	1.11	0.11	U29513	KRAB-zinc finger protein 79; Kruppel type zinc finger putative transcriptional repressor; associates with RB in vitro	Hematopoietic cells; others?
0.001	1.03	0.03	0.21	0.14	1.04	0.25	ET62444	Sox4; transcription factor in the Sox gene family with separable DNA-binding and transactivation domains	Thymus, bone marrow, and gonads
PRIMARY RESPONSE GENES - LIVER									
0.002	0.91	0.20	0.22	0.20	1.15	0.14	L24118	TNF-inducible; primary response gene B94	Liver (during development) and monocytes (postnatally)
0.002	1.19	0.32	0.00	0.00	1.27	0.33	X83601	PTX3, entraxins; include C reactive protein (CRP) and serum amyloid P component (SAP) which are prototypic acute phase reactants that serve as indicators of inflammatory reactions.	Liver, skeletal muscle and heart

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.002	1.09	0.31	0.00	0.00	1.39	0.39	M31419	Interferon-activatable gene (204); mediates antimicrobial, immunomodulary and cell growth-regulatory activities of interferons; increased up to 75-fold by alpha-interferon treatment	Nucleoi
PRIMARY RESPONSE GENES - OTHER TISSUES									
<0.001	1.11	0.17	0.09	0.15	1.00	0.17	D13695	ST2L, primary response gene; specifically induced by growth stimulation; highly similar to IL1 receptor type 1	T cells
0.005	1.28	0.46	0.00	0.00	1.40	0.39	U34245	Fos-related antigen-1 (Fra-1)	Spleen
<0.001	1.04	0.04	0.00	0.00	1.14	0.21	Y08026	Immunity associated protein 38; inducible by malaria	Spleen
0.001	1.35	0.36	0.00	0.01	1.23	0.20	M81077	TAL2	T cells
<0.001	1.03	0.14	0.00	0.00	1.10	0.09	U19463	Zinc finger protein A20; activated by T cell acute lymphoblastic leukemia; helix-loop-helix DNA binding protein	Lymphocytes
0.1	1.27	0.24	0.00	0.00	1.40	1.23	L15435	4-1BB ligand, inflamatory response; member of the TNF family; important for the generation of antiviral CD8 T cell responses	T cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
<0.001	1.18	0.18	0.00	0.00	1.03	0.16	M88242	Glucocortoid-regulated inflammatory cyclooxygenase; prostaglandin G/H synthase, putative mediator of inflammation; mRNA and protein rise dramatically in response to growth factors, cytokines, and oncogene activation; suppressed by glucocorticoid hormone	Fibroblasts and human monocytes
<0.001	1.07	0.12	0.00	0.00	1.16	0.09	L38281	Immune-responsive gene 1 (Irg1); activated by bacterial LPS treatment	Macrophages
0.001	1.15	0.32	0.02	0.03	1.31	0.28	K02785	Gene induced by PDGF with some homology to c-fos	Unknown
INTRACELLULAR TURNOVER									
0.002	0.95	0.10	0.43	0.27	1.37	0.11	X92664	Ubiquitin-conjugating enzyme UbcM2 (E2); nonlysosomal protein degradation system; histone ubiquitination alters chromatin structure	Liver, skeletal muscle (ubiquitous)
0	1.11	0.11	0.00	0.00	1.02	0.10	X71978	Ft1, a novel gene related to ubiquitin-conjugating enzymes; deletion leads to partial syndactyly of the limbs and thymic hyperplasia, suggesting impaired programmed cell death	

P	CR	std	CON	std	SW	std	GenBank	Description	Location
CHROMATIN STRUCTURE									
0.028	1.33	0.95	0.00	0.00	1.79	0.49	L04141	Histone H1 subtype (H1e); chromatin structure	Liver (ubiquitous)
0.000	1.74	0.30	0.00	0.00	1.08	0.24	J03482	Histone H1; chromatin structure	Ubiquitous
0.000	1.69	0.18	0.02	0.03	1.09	0.27	ET62262	Histone H1b; chromatin structure	Ubiquitous
0.030	1.41	0.26	0.34	0.53	1.10	0.24	ET62908	Histone H2B; chromatin structure	Liver (ubiquitous)
0.038	1.20	0.27	0.00	0.00	0.79	0.70	U62675	Histone H3.2-616, and histone H2b-616; chromatin structure	Liver (ubiquitous)
0.006	1.08	0.12	0.15	0.18	1.12	0.40	U62672	Histone H3.1-D (H3-D) and histone H4-D (H4-D) genes; chromatin structure	Ubiquitous
0.024	1.22	0.82	0.00	0.00	1.36	0.11	X16495	Histone H2A; chromatin structure	Ubiquitous
<0.001	1.07	0.13	0.00	0.00	1.19	0.13	U62669	Histone H3.2-F (H3-F), histone H2a.1-F (H2a-F), histone H2b-F (H2b-F); chromatin structure	Ubiquitous
0.034	1.11	0.15	0.30	0.52	1.16	0.21	X56044	Htf9-c; structural similarity with yeast and bacterial nucleic acid-modifying enzymes; activated at the G1/S transition, maximum and S phase; down in growth arrested cells	Liver (ubiquitous)
0.009	1.06	0.11	0.28	0.48	1.44	0.21	X56690	Homologous to Drosophila HP1 gene; modifs chromatin, rendering heritable changes in gene expression; activates or silences genes	Ubiquitous during development

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.025	1.51	0.35	0.27	0.47	0.99	0.36	X92842	SURF-6; involved in a nucleolar ribosome maturation; housekeeping	Nucleolus (ubiquitous)
CELL CYCLE / CELL DIVISION									
0.022	1.11	0.17	0.00	0.00	0.82	0.60	X66285	HC1 gene; mouse gene homologous to the E5 ORF from bovine papillomavirus type 1; transforms immortalized rodent cells.	
0.000	1.09	0.27	0.00	0.00	1.54	0.14	ET62229	Wnt10b; developmental regulation of cell growth and differentiation in certain adult mammalian tissues	Developing limbs, face and skin of embryos and in adult
0.003	1.66	0.58	0.00	0.00	1.11	0.22	ET61747	Citron; Rho (controls actin structures) target protein; role in cytokinesis	Ubiquitous
0.001	1.93	0.38	0.00	0.01	1.12	0.45	Y00848	Int-2 (FGF-3); expressed in embryonic development	Early embryogenesis; discrete regions during development; not reported in adult
0.002	1.46	0.17	0.47	0.20	1.08	0.17	X61940	Mitogen-activated protein kinase phosphatase (MKP-1/3CH134/ERP1); serum growth factor-induced immediate early gene; dephosphorylates MAP kinase	Liver parenchymal cells, vascular smooth muscle, others
<0.001	1.08	0.07	0.00	0.00	1.05	0.20	Z72000	BTG3; negative control of cell cycle	Fibroblast, brain
<0.001	1.13	0.11	0.03	0.05	1.04	0.16	X07540	C-abl; c-Abl; a nonreceptor tyrosine kinase; appears to play a role in cell cycle progression, cell proliferation and differentiation	Liver, B cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.006	1.02	0.02	0.00	0.00	0.92	0.45	U10440	G1 cyclin-Cdk protein kinase inhibitor p27, cell cycle; cyclin-dependent kinase inhibitor p27 (Kip1)	Ubiquitous
0	1.08	0.17	0.00	0.00	1.29	0.21	ET61628	Phosphoinositide 3-kinase (regulatory subunit p85alpha); plays critical roles in cell growth, differentiation, survival, and vesicular transport	Liver
0.002	1.48	0.47	0.00	0.00	1.26	0.23	ET61257	Map Kinase Kinase Kinase (MEKK 1) ; MEK kinases (MEKKs) are serine-threonine kinases that regulate sequential protein phosphorylation pathways involving mitogen-activated protein kinases (MAPKs), including members of the Jun kinase (JNK) family.	Ubiquitous
0.002	1.10	0.41	0.00	0.00	1.25	0.22	U85608 (was U11548)	Mitogen-activated protein kinase (MAPK); signal transduction; important in cell proliferation, differentiation, and apoptosis; induced by epidermal growth factor; activation of MAPK induces c-Fos and c-Jun; CR reduces the age related decline in MAPK activation	Liver (15 times higher in fetal than adult); ubiquitous '
0.002	1.09	0.31	0.00	0.00	1.39	0.39	M31419	Interferon-activatable gene (204); mediates antimicrobial,	Nucleoi

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								immunomodulary and cell growth-regulatory activities of interferons; increased up to 75-fold by alpha-interferon treatment	
DNA REPLICATION / REPAIR									
0.029	1.36	0.37	0.48	0.34	1.27	0.24	D13544	Primase small (p49) subunit; cell proliferation; DNA replication	Liver (ubiquitous)
0.025	1.24	0.37	0.45	0.16	1.14	0.25	X74351	XPAC (Xeroderma Pigmentosum group A Correcting protein); nucleotide excision DNA repair	Ubiquitous
0.001	1.03	0.29	0.00	0.00	1.27	0.30	ET62746	Brca2 gene; familial breast cancer susceptibility gene; important in DNA double-strand break repair (DSBR) and DNA damage-induced cell-cycle checkpoint activation	Ubiquitous
<0.001	1.17	0.13	0.13	0.14	1.07	0.19	X58472	KIN17, DNA-binding, nuclear protein, upregulated in response to UV and ionizing radiation; accumulated in the nucleus of proliferating fibroblasts; overexpression inhibits progression into S phase	Ubiquitous
0.009	1.02	0.03	0.17	0.14	0.85	0.37	ET63479	MLH1; DNA mismatch repair gene; function in mutation avoidance; cell cycle checkpoint control; cytotoxicity. of various DNA-damaging agents; transcription-coupled nucleotide excision repair.	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
APOPTOSIS									
0.005	1.07	0.19	0.33	0.30	1.36	0.23	Z37110	Cyclin G; augments apoptosis; target gene of P53	Liver
0.000	1.00	0.09	0.42	0.05	1.12	0.04	ET63241	Apopain precursor (LICE; caspase-3; YAMA protein); cysteine protease; mediator of apoptosis; processes precurser IL-1; PARP-cleaning	Liver, neurons, lung, kidney, spleen, lymphocytes
<0.001	1.14	0.13	0.05	0.09	1.06	0.11	X58876	Mdm2 is a P53 specific ubiquitin ligase; promotes the ubiquitination and proteasome-dependent degradation of p53; immediately after cellular stress, MDM2 ability to bind to p53 is blocked, preventing MDM2-mediated degradation, P53 levels rise causing cell cycle arrest or apoptosis	Liver
<0.001	1.12	0.15	0.00	0.00	1.08	0.24	L22472	Bax alpha; Bcl-2-family protein with pro-apoptotic activity; can form channels in lipid membranes	Liver
0.001	1.21	0.28	0.00	0.00	1.16	0.22	L31532	Bcl-2-beta; suppresses programmed cell death	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.050	1.25	0.15	0.47	0.50	1.01	0.07	U48804	Zn-finger protein Pw1/Peg3; activates NFkappaB; regulator of TNF response; induced during p53/c-myc-mediated apoptosis; Pw1/Peg3 with Siah1a induces apoptosis independently of p53; inhibiting Pw1/Peg3 activity blocks p53-induced apoptosis.	Ubiquitous
0	0.91	0.19	0.00	0.00	1.08	0.06	ET61211	RNA-dependent EIF-2 alpha kinase; double-stranded (ds) RNA-dependent protein kinase (PKR); key mediator of antiviral effects of interferon (IFN); active player in apoptosis.	Ubiquitous
0	1.11	0.11	0.00	0.00	1.02	0.10	X71978	Ft1, a novel gene related to ubiquitin-conjugating enzymes; deletion leads to partial syndactyly of the limbs and thymic hyperplasia, suggesting impaired programmed cell death	
SERUM PROTEINS / SECRETED PROTEINS									
0.000	1.28	0.19	0.11	0.19	0.95	0.09	V00743	Alpha-fetoprotein (AFP); main component of mammalian fetal serum; synthesized by visceral endoderm of the yolk sac and by fetal liver; blood level decreases after birth; synthesis reactivated in liver tumors	Liver (fetal & adult)

P	CR	std	CON	std	SW	std	GenBank	Description	Location
<0.001	1.10	0.09	0.07	0.12	1.07	0.16	M16395	Alpha-fetoprotein (AFP); main component of mammalian fetal serum; synthesized by visceral endoderm of the yolk sac and by fetal liver; blood level decreases after birth; synthesis reactivated in liver tumors	Liver (fetal & adult)
0.018	1.14	0.13	0.38	0.42	1.30	0.26	X03479	Serum amyloid A (Saa) 3; serum protein; major acute phase protein	Liver
0.049	1.15	0.64	0.57	0.18	1.71	0.35	ET63455	Serum amyloid A-4 protein (Saa4); a minor, normal high- density lipoprotein (HDL, apolipoprotein); acute-phase apolipoprotein; induced by trauma and inflammation; normally rapidly catabolized; degraded by secreted or cell-associated neutral proteases generated by macrophages	Epithelial cells in a variety of tissues including liver
0.008	1.57	0.50	0.14	0.25	1.00	0.25	V00829	Kallikrein; serine protease; generates proinflammatory kinins; processes peptides	Liver
0.002	1.27	0.40	0.00	0.00	1.35	0.31	X61597	Kallikrein-binding protein; tissue kallikrein regulation; serine proteinase inhibitor superfamily	Liver, lung, thymus
EXTRACELLULAR MATRIX / CELL ADHESION									
0.002	1.60	0.32	0.03	0.05	1.17	0.41	Z50147	Cell adhesion regulator; extracellular matrix protein	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.000	1.34	0.27	0.05	0.08	0.95	0.11	X06115	E-cadherin; cell-cell adhesion; cell surface glycoprotein; transmembrane protein	Liver (epithelial cells)
0.001	0.94	0.16	0.42	0.06	1.26	0.14	ET62381	K-cadherin/cadherin-6; present at external cell surface at cell-cell contact sites; calcium-dependent cell adhesion molecules	Cerebral cortex in neonatal mice; newly formed epithelium of the renal vesicle; proximal renal tubules; CD4+ CD8+ thymocytes
0.006	1.05	0.09	0.35	0.31	1.06	0.05	U69137	T2-cadherin; calcium-binding membrane glycoprotein; cell adhesion molecule	Thymocytes; developing testis and retina
0.003	1.08	0.13	0.09	0.15	0.99	0.34	X77557	cadherin 11(cad11); calcium-dependent mesenchymal cell adhesion molecule	Mesoderm surrounding organs; Developing somites;
0.004	1.56	0.22	0.25	0.29	1.17	0.34	X67783	Vascular cell adhesion molecule-1 (VCAM-1); immunoglobulin gene superfamily; transmembrane	Liver
<0.001	1.29	0.29	0.01	0.03	1.22	0.19	X66976	Collagen alpha 1 type VIII; extracellular matrix: component of basal laminae	Epithelial, endothelial, and mesenchymal cells in newborn mouse tissue
0.004	1.37	0.47	0.00	0.00	1.32	0.34	Z35166	Collagen IV alpha 3 chain; extracellular matrix; component of basal laminae	Liver
0.012	1.08	0.18	0.30	0.42	1.51	0.36	Z35168	Collagen IV alpha 5 chain; collagen; extracellular matrix	Liver
0.006	1.11	0.45	0.00	0.00	1.42	0.39	L02918	Procollagen type V alpha 2	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	0.97	0.11	0.14	0.25	1.48	0.29	X66402	Stromelysin 1; extracellular matrix-degrading metalloproteinase	Liver, stromal cells
0.009	1.30	0.57	0.00	0.00	1.46	0.43	U08210	Tropoelastin; elastic fibers in vessel walls and other tissues consist of cross-linked tropoelastin in association with several microfibrillar protein	Vessel
<0.001	1.12	0.16	0.00	0.00	1.15	0.13	X16490	Plasminogen activator inhibitor 2; serine protease inhibitor; inactivates urokinase-type plasminogen activator and regulates degradation of the extracellular matrix; one form is cytoplasmic the other is translocated into the endoplasmic reticulum, glycosylated and secreted	Liver; mainly expressed in the skin, bone-marrow, spleen, lung, thymus, and urinary bladder
0.010	1.21	0.41	0.32	0.29	2.11	0.63	D13509	Pancreatitis-associated protein (PAP); C-type lectin; adhesion protein; binds laminin; may be important in liver cell differentiation/proliferation; adhesion molecule for hepatocytes	Liver (ductular cells), pancreas, small intestine
0.014	1.31	0.46	0.13	0.23	1.66	0.59	ET63188	Fibroblast activation protein; cell-surface glycoprotein; member of the serine protease family; expressed at sites of tissue remodelling.	Fibroblasts

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.017	1.61	0.70	0.00	0.00	1.45	0.59	X75636	Iduronato-2-sulfatase (IDS); degrades heparin sulfate and dermatan sulfate in lysosomes; deficiency causes fatal lysosomal storage disorder, mucopolysaccharidosis type II (the glycosaminoglycans heparin sulfate and dermatan sulfate accumulate); part of proteoglycans which bind, help package and store secretory molecules; function in cell adhesion and basal lamina formation	Ubiquitous
TRANSPORT / SECRETION									
0.010	1.15	0.14	0.31	0.37	1.09	0.16	ET63248	RAN binding protein 1 (RANBP1); RAN-specific GTPase-activating protein; required for nucleocytoplasmic transport of many types of cargo	Ubiquitous
0.013	0.99	0.16	0.25	0.41	1.15	0.15	D87900	ARF3; ADP-ribosylation factor; involved in formation of coated vesicles	Ubiquitous
0.001	1.04	0.07	0.38	0.16	1.12	0.12	U19521	Vesicle transport protein (munc-18c)	Ubiquitous
0.001	1.12	0.15	0.43	0.09	1.06	0.13	X14972	Alpha-adaptin; adaptor complex components; link clathrin to coated vesicle receptors	Liver and brain

P	CR	std	CON	std	SW	std	GenBank	Description	Location
<0.001	1.05	0.07	0.00	0.01	1.04	0.17	Z22821	Rab23; Ras-related small GTPase; protein trafficking; central regulatory elements of the intracellular transport machinery; regulate vesicle docking and fusion, organelle dynamics	Liver regeneration leads to differential regulation of some Rabs; other cells
0.003	1.23	0.28	0.00	0.00	1.28	0.45	D29797	Syntaxin 3A, IER vesicular transport, membrane fusion	Liver
<0.001	1.08	0.09	0.01	0.02	1.13	0.11	X66449	Calcyclin, also called S100A6; calcium binding protein; mucus secretion.	Epithelial cells and fibroblasts of liver; breast, heart, intestine, kidney, ovary, placenta, stomach, thymus, and uterus; high levels of expression in epithelial lining the gastrointestinal, respiratory and urinary tracts
0.001	1.21	0.18	0.00	0.00	1.12	0.29	U96700	Serine proteinase inhibitor 6 (SPI6); not secreted, remains in the endoplasmic reticulum; intracellular function unclear	Lymphocytes; endothelial and epithelial cells; platelets
0.013	1.10	0.15	0.00	0.00	1.05	0.58	L39373	N-acetylglucosaminyltransferase III (Mgat3); transfers the bisecting GlcNAc to the core of complex, N-linked carbohydrates	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	0.97	0.15	0.48	0.11	1.08	0.07	U58513	Rho kinase (p160, ROCK-2); Rho is a small GTPase; serine/threonine coiled coil- forming protein kinase; downstream targets include LIM-kinase 1, which phosphorylates cofilin, an actin-depolymerizing factor; regulates actin cytoskeletal reorganization; Rho activity enhances secretion; phosphorylation of myosin light chain and moesin may prevent pathologic platelet activation during atherogenesis.	Ubiquitously expressed except in the brain and muscle
0.009	2.03	0.56	0.24	0.41	1.11	0.39	U96724	Phosphatidylinositol transfer protein alpha (Pitpn); cytosolic protein binds phosphatidylinositol and transfers it between membranes; mutant in this gene (the mouse vibrator mutation) causes an early-onset progressive action tremor, degeneration of brain stem and spinal cord neurons, and juvenile death.	Ubiquitous
0.001	1.27	0.13	0.07	0.12	0.94	0.31	X78304	Signal recognition particle (SRP9); cytoplasmic ribonucleoprotein; synthesis and translocation of secreted proteins	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	1.01	0.08	0.15	0.25	1.22	0.18	ET62525	Polypeptide N-acetylgalactosaminyltransferase-T4 (polypeptide GalNAc transferase-T4; ppGalTase-T4); fourth member of the mammalian UDP-GalNAc; Golgi-like localization; 4 GalNAc-transferase controls the initiation of mucin-type O-linked protein glycosylation, in which N-acetylgalactosamine is transferred to serine and threonine amino acid residues	Wide expression pattern; detected in embryonic tissues, as well as adult sublingual gland, stomach, colon, small intestine, lung, cervix, and uterus; lower levels detected in kidney, liver, heart, brain, spleen, and ovary
0.017	1.07	0.06	0.25	0.44	0.97	0.11	X14926	Calreticulin; endoplasmic reticulum chaperone; also functions in calcium storage and signaling, and cell attachment; nuclear matrix component	
0.017	1.61	0.70	0.00	0.00	1.45	0.59	X75636	Iduronato-2-sulfatase (IDS); degrades heparin sulfate and dermatan sulfate in lysosomes; deficiency causes fatal lysosomal storage disorder: mucopolysaccharidosis type II (the glycosaminoglycans heparin sulfate and dermatan sulfate accumulate); part of proteoglycans which bind, help package and store secretory molecules; function in cell adhesion and basal lamina formation	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
TRANSLATION									
0.011	1.04	0.05	0.29	0.28	1.12	0.31	U28419	Translation initiation factor eif-4C homologue	Ubiquitous
0.000	1.48	0.24	0.00	0.00	1.05	0.08	X57960	Ribosomal protein L7; incorporated into 60 S subunit	Ubiquitous
0.013	1.17	0.22	0.37	0.29	1.04	0.18	M29015	Ribosomal protein L7 (rpL7); incorporated into 60 S subunit	Ubiquitous
<0.001	1.06	0.05	0.00	0.00	1.18	0.25	K02060	Ribosomal protein L32	Ubiquitous
0.001	1.27	0.13	0.07	0.12	0.94	0.31	X78304	Signal recognition particle (SRP9); cytoplasmic ribonucleoprotein; synthesis and translocation of secreted proteins	Ubiquitous
TRANSCRIPTION									
0.026	1.20	0.18	0.34	0.35	1.36	0.48	X74040	Mesenchyme fork head-1 (MFH-1) transcription factor	H+J509 hepatocytes
0.016	1.32	0.28	0.31	0.26	1.08	0.37	ET61028	ARE Binding Protein (AREC3)	Many cell-types during development; muscle in adult
0.005	1.16	0.04	0.00	0.00	0.77	0.46	ET62446	Sox12; transcription factor; Sox family plays important role in development	Developing embryos
0.018	1.19	0.27	0.39	0.32	1.14	0.18	X55781	Pax2 transcription factor; paired box family (homologous to Drosophila segmentation genes)	Developing embryo excretory and CNS

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.032	1.16	0.18	0.50	0.35	1.26	0.09	ET62078	Putative transcription factor (tbx4); T-box DNA binding domain; putative roll in inductive interactions during embryogenesis	Many locations in embryo during development
0.003	1.06	0.13	0.48	0.25	1.31	0.11	X75018	Id4; CD44; dominant negative regulators of bHLH transcription factors; differentiation in cellular systems including myogenesis, neurogenesis and haematopoiesis; adipocyte differentiation	Embryogenesis, up-regulated between day 9.5 and 13.5 of gestation; adult highest expression testis, brain and kidney; also in liver; adipocytes, astrocytes, muscle cells and others
0.009	1.56	0.64	0.00	0.00	1.47	0.44	L28167	Zinc finger protein, the Kruppel-associated box (KRAB); similar to profilaggrin (expressed in differentiating epidermal cells)	Liver, lens, heart, kidney, spleen, brain of newborn mice
0.003	1.17	0.37	0.10	0.16	1.21	0.18	U13878	Neural-restrictive silencer factor (NRSF/REST); transcription factor; represses expression of neuronal genes including mAChR, SCG-10 and type II sodium channel genes; recruits mSin3 and histone deacetylase	Many nonneuronal cells and tissues
<0.001	1.09	0.12	0.11	0.13	1.09	0.09	X89264	Zinc-finger protein Zfp-37; transcription factor (putative); peroxisome proliferator responsive; contains Kruppel-associated box	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.018	1.18	0.62	0.08	0.07	1.06	0.08	U15443	C-ros (c-rosy; embryonic development; tyrosine kinase catalytic domains; expressed in neoplastic and fetal tissues	Neoplastic and fetal tissues
0.003	1.24	0.40	0.00	0.00	1.25	0.32	X59251	Hox-7; transcription factor; early stage of eye developmental regulation in embryo	Embryogenesis
0.001	1.12	0.27	0.10	0.18	1.17	0.12	M28449	Hox-1.7; homeobox; transcription factor	Embryogenesis
0.01	1.53	0.65	0.00	0.00	1.50	0.46	X56182	Mvf-5; myogen factor 5; transcription factor; helix-loop-helix family	Embryonic liver and heart
0.047	1.45	0.81	0.00	0.00	1.42	0.72	X13538	Hox-1.4; transcription factor	Embryonic spinal cord and adult testis
0.001	1.23	0.23	0.03	0.05	1.11	0.26	X60034	Hox-4.9; homeobox; transcription factor	Neurogenesis
0.002	1.27	0.23	0.00	0.00	1.14	0.38	X80339	Six1; homeobox; development of limb tendons	Skeletal and smooth muscle
0.024	1.56	0.52	0.00	0.00	1.49	0.83	D00925	Transcription factor S-II-related protein; transcription elongation factor	Liver
0.003	1.17	0.15	0.13	0.22	0.88	0.27	X67719	CREBcAMP-responsive-element binding protein	Ubiquitous
0.009	1.50	0.67	0.00	0.00	1.34	0.29	X60136	Sp1; transcription factor; zinc finger protein	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
<0.001	1.16	0.03	0.09	0.15	1.02	0.07	X80508	Yes-associated protein (YAP65); transcription activator	Ubiquitous
<0.001	1.07	0.06	0.00	0.00	095	0.13	X76858	Phi AP3, nuclear factor; DNA binding transcription factor; inactivates adjacent enhancer function; GLI-Kruppe related; cell-cycle regulated	Ubiquitous
0.002	1.99	0.65	0.00	0.00	1.08	0.19	Y12783	Ring1B; interacts directly with the repressor domain of M33; M33 is a transcription factor implicated in mesoderm patterning in the mouse; in Drosophila, homologue genes maintain transcriptional repression of developmental genes including homeotic genes	Expression distribution not reported
0.003	1.08	0.17	0.17	0.29	1.41	0.32	X55315	CAAT-box DNA binding protein subunit A (NF-YA)	Ubiquitous
0	1.36	0.25	0.00	0.00	1.02	0.04	X15842	C-rel; encodes a member of the Rel/nuclear factor (NF)-kappaB family of transcriptional factors	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.011	1.06	0.08	0.38	0.33	1.04	0.10	ET63161	Alternative splicing factor (ASF); recruits basal splicing factors during spliceosome assembly	Ubiquitous (?)
0.001	1.04	0.12	0.15	0.26	1.07	0.07	Y08260	CPEB protein; RNA binding protein that interacts with the maturation-type cytoplasmic polyadenylation element to promote polyadenylation and translational activation	Ubiquitous
0.004	1.14	0.19	0.00	0.00	1.02	0.44	X91656	Srp20 gene; splicing factor belonging to the highly conserved family of SR proteins; multiple roles in the regulation of constitutive and alternative splicing	Ubiquitous
MEMBRANE PROTEIN									
0.003	1.08	0.17	0.00	0.00	1.47	0.51	M17376	Alpha-1-acid glycoprotein I (AGP-1); membrane protein	Liver
0.001	1.48	0.19	0.00	0.00	0.97	0.37	M75875	MHC class I T3-d gene; H-2-d haplotype; beta-2-microglobulin associated protein; cell surface glycoprotein; class I antigen	Unknown
0.036	1.09	0.10	0.44	0.38	1.09	0.23	J03298	Major histocompatibility complex DO beta gene	Ubiquitous
0.001	1.76	0.14	0.01	0.01	1.02	0.47	D90146	MHC gene Q8/9d Qa-2,3 class I antigen	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.001	1.12	0.29	0.00	0.00	1.16	0.18	U06662	59-kd oncofetal antigen; antigens present on the surface of all major classes of rodent tumors	Fetal antigen; not reported in adult tissues
0.008	1.07	0.73	0.00	0.00	1.73	0.16	X61576	Connexin 43; .[??] gap junction proteins; contain ion exchange channels that generate signals throughout the tissue	Liver, heart, bone, skin, etc.; Mol Carcinog 1996 Aug;16(4):203-12
0.024	1.41	0.69	0.00	0.00	1.45	0.61	M91243	Connexin family of gap junction (cell-to-cell channels) proteins (Cx50); likely IS lens fiber protein	Ubiquitous
0.023	1.38	0.74	0.00	0.00	1.30	0.40	X54424	Gamma adaptin; major component of adaptor; the protein complex links clathrin to transmembrane proteins in coated pits and vesicles	Liver (ER)
0.005	1.43	0.52	0.00	0.00	1.37	0.38	U49185	Occludin; occludin is a transmembrane protein located at tight junctions and is known to interact with other tight junction proteins	Liver
0.027	1.28	0.32	0.60	0.13	1.43	0.37	M81591	CD10 neutral endopeptidase 24.11 (CD10/NEP); cell surface metalloproteinase; activation marker for mononuclear cells; peptide mediated signal	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								transduction; inactivates numerous endogenous peptides in the brain, kidney, and lung in vivo	
0.009	1.06	0.75	0.00	0.00	1.93	0.43	Z22216	Apolipoprotein C2 (APOC2); required for lipolysis of triglycerides by lipoprotein lipase	Fetal liver, adult liver, intestine and peritoneal macrophages
0.026	1.09	0.03	0.50	0.19	0.93	0.27	V00834	MHC class II H2-IE-alpha	B cells, IgE
0.003	1.16	0.07	0.46	0.13	0.90	0.21	X68061	Beta-2-microglobulin; membrane protein; 45,000 MW HLA antigen	Liver (hepatocytes)
0.002	0.99	0.29	0.00	0.00	1.38	0.36	M23383	Glucose transporter 2	Liver
0.038	1.24	0.60	0.22	0.28	1.08	0.12	ET63259	Cea14 gene (carcinoembryonic antigen family members); unknown function; member of the immunoglobulin superfamily	Many cea genes expressed in fetal liver
0	1.08	0.15	0.18	0.17	1.32	0.16	ET63260	Cea15 gen (carcinoembryonic antigen family members); unknown function; member of the immunoglobulin superfamily	Many tea genes expressed in fetal liver
0.001	1.04	0.04	0.22	0.12	1.16	0.28	ET63261	Cea16 gene (carcinoembryonic antigen family members); unknown function; member of the immunoglobulin superfamily	Many cea genes expressed in fetal liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
METABOLISM/ENERGY GENERATION									
0.003	0.97	0.05	0.18	0.26	1.34	0.33	U00932	Glutamine; fructose-6-phosphate amidotransferase (GFAT); rate-limiting enzyme in hexosamine synthesis	Liver
0.016	1.10	0.16	0.33	0.41	1.24	0.21	D21826	CMP-N-acetylneuraminic acid hydroxylase; ganglioside expression	Liver
0.001	1.08	0.31	0.00	0.00	1.58	0.32	X98792	Prostaglandin synthase cyclooxygenase Down in CR	Liver
0.002	1.23	0.28	0.01	0.02	1.42	0.41	M29395	Orotidine-5'-monophosphate decarboxylase; conversion of orotidine 5'-monophosphate to UMP; UMP biosynthetic pathway	Liver
<0.001	1.01	0.03	0.01	0.01	1.11	0.10	X72959	Nat3 gene for N-acetyltransferase	Liver
0.001	1.10	0.13	0.06	0.10	1.20	0.30	J04947	ACE; angiotensin-converting enzyme	Liver
<0.001	1.16	0.02	0.04	0.08	1.02	0.13	L09105	Glucose phosphate isomerase	Ubiquitous
0.039	1.39	0.70	0.00	0.00	1.56	0.80	X14489	Thymidylate synthase (TS)	Ubiquitous (all proliferating cells)
0.005	1.37	0.42	0.00	0.00	1.54	0.51	U34071	Alpha-galactosidase A; lysosomal enzyme	Ubiquitous (most cases)
0.004	1.08	0.20	0.51	0.17	1.11	0.03	J00355	Alpha-amylase-1 (Amy-1 A); glycogen digestion and	Liver and salivary glands

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								mobilization	
0	1.10	0.09	0.29	0.05	1.20	0.19	X07888	3-hydroxy-3-methylglutaryl coenzyme A reductase; key regulatory enzyme for cholesterol biosynthesis	Liver
ION CHANNELS/PUMP									
0.000	1.42	0.20	0.00	0.00	0.95	0.14	ET61677	Epithelial sodium channel alpha subunit	Liver
0.044	1.33	0.44	0.35	0.34	1.07	0.33	U03723	AKR voltage-gated potassium-channel (KCNA4)	Ubiquitous
0.039	1.24	0.23	0.45	0.34	1.09	0.31	M30441	Potassium channel gene (MK3)	Ubiquitous
0	1.27	0.05	0.13	0.13	1.07	0.13	ET61590	Putative capacitative calcium entry channel (Trp6); involved in calcium entry secondary to activation of receptors coupled by the Gq class of G protein	Brain, kidney, heart and lung, no trp message detected in liver
0.002	1.19	0.35	0.06	0.10	1.43	0.34	ET61440	Trp-related protein 3; cation channel; essential for agonist-activated capacitative Cat+ entry; putative subunits of CCE channels	Endothelium
NUCLEAR RECEPTORS									
0.016	1.38	0.67	0.07	0.13	1.28	0.26	X07751	Thyroid hormone receptors	Liver
0.003	1.24	0.31	0.13	0.16	1.23	0.26	X04435	Glucocorticoid receptor	Liver
<0.001	1.20	0.23	0.03	0.05	1.11	0.15	X74134	COUP-TF1; steroid hormone receptor;	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								transcription factor	
0.01	1.13	0.12	0.00	0.00	1.21	0.61	X76653	Apolipoprotein regulatory protein1 (ARP-1); member of the COUP-family of steroid hormone orphan receptors	Liver, lung, kidney
0.049	0.90	0.55	0.35	0.04	1.40	0.43	X59411	Androgen receptor	Sex glands, liver, brain, pituitary, heart, kidney, bone
CYTOKINE/GROWTH FACTOR									
0.003	1.10	0.23	0.11	0.18	1.61	0.45	X56413	Transforming growth factor-beta2 (TGFbeta2); cell proliferation	Liver stellate cells
0.012	1.11	0.23	0.35	0.32	1.05	0.06	ET62118	Keratinocyte growth factor/fibroblast growth factor-7 precursor (mKGF)	Liver epithelial cells
0.001	1.38	0.08	0.00	0.00	0.94	0.40	Z29532	Follistatin; binds and inactivates activin; up-regulated by mediators of inflammation; control of the. inflammatory cascade	Liver
0.000	1.41	0.13	0.11	0.17	0.94	0.25	M28587	Alpha leukocyte interferon (MulFN-alpha A); inhibition of cell proliferation	Ubiquitous
0.015	1.31	0.23	0.44	0.35	1.04	0.13	V00755	Interferon beta (type 1); growth factor; T helper cell differentiation factor; antiviral; modulates immune responses to	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								foreign and self-antigens	
0.002	1.10	0.10	0.48	0.17	1.15	0.16	M30644	Basic fibroblast growth factor (Fgfb; FGF-2); potent trophic effects on neurons, glia and endothelial cells; mitogen, differentiation and survival factors, angiogenic factor; levels are markedly elevated after liver injury; stimulates hepatocyte proliferation and migration at the wound front	Endothelial cells (vascular); pituitary; peritoneal mesothelial cells; astrocytes; leukocytes
<0.001	1.12	0.04	0.09	0.15	1.12	0.21	X53257	NT-3 gene for neurotrophin-3; secreted protein; binds high affinity receptor trk C; postnatal development?	Liver parenchymal cells, olfactory bulb, cerebellum, septum, hippocampus; thymus, heart, diaphragm, pancreas, spleen, kidney, adrenal
0.001	1.07	0.07	0.10	0.17	1.13	0.23	J00424	Interferon-beta	Liver
0.001	1.10	0.11	0.02	0.02	1.18	0.38	U96386	Activin beta E subunit, member of TGF-beta superfamily	Liver
0.005	1.24	0.41	0.00	0.00	1.38	0.39	X69620	Inhibin beta-B subunit; activins are dimeric proteins, members of the transforming growth factor beta (TGF-beta) gene	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								superfamily, consisting of beta-subunits of inhibin (betaA and betaB)	
0.003	2.25	0.40	0.18	0.32	1.19	0.55	X99572	C-fos-induced growth factor (FIGF); secreted dimeric protein member of the , platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF) family; mitogenic and morphogenic activity on fibroblasts.	Endothelial cells, expressed in many tissues (including liver) during embryonic development
0.003	1.11	0.10	0.26	0.26	1.17	0.22	U07982	Preproendothelin-1; induces smooth muscle alpha-actin expression; induced in stellate and endothelial cells of liver after injury	Vascular wall (endothelial cells, arterial smooth muscle cells, select epithelial cells); kidney, lung, trachea; liver (nonparenchymal cells, predominately in sinusoidal endothelial cells)
SIGNAL TRANSDUCTION									
0.004	1.30	0.21	0.30	0.29	1.09	0.19	X82320	Stathmin; cytosolic phosphoprotein participating in relay and integration of intracellular signaling pathways involved	Ubiquitous

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								in control of cell proliferation, differentiation, and other activities	
0.041	1.19	0.25	0.41	0.28	1.13	0.40	X13664	N-ras; key component of growth signaling pathways; transmits membrane receptor kinase signals; GTP-binding switch protein	Liver, wide tissue distribution
0.002	1.46	0.17	0.47	0.20	1.08	0.17	X61940	Mitogen-activated protein kinase phosphatase (MKP-1/3CH 134/ERP1); serum growth factor-induced immediate early gene; dephosphorylates MAP kinase	Liver parenchymal cells, vascular smooth muscle, others
0.004	1.17	0.48	0.00	0.00	1.09	0.15	S45828	Serine/threonine/tyrosine protein kinase (Nek1); related to the NIMA (a protein kinase which controls initiation of mitosis in Aspergillus nidulans)	All organs examined
0.001	1.09	0.27	0.10	0.15	1.23	0.22	U65313	Ras-GTPase-activating SH3-domain binding protein (G3BP); essential for Ras signaling	Ubiquitous
0.013	1.02	0.03	0.00	0.00	1.02	0.57	M63658	G protein beta-subunit	Brain, liver, blood cell
0.003	1.09	0.11	0.03	0.05	1.25	0.45	U38501	G protein alpha i1 subunit	Liver; cerebral cortex; pancreatic acinar cells; white adipose tissue;

P	CR	std	CON	std	SW	std	GenBank	Description	Location
									others
0.010	1.32	0.28	0.24	0.41	1.06	0.11	AA162130	SUMO-1 activating enzyme subunit 1 (SAE1); one subunit of a dimer that conjugates SUMO-1 (a small ubiquitin-like protein) to other proteins; SUMO-1 modification of I Kappa B alpha takes place on the same residues used for ubiquitination; blocks NF kappa B-dependent transcriptional activation	Ubiquitous
0.003	1.11	0.50	0.00	0.00	2.16	0.55	ET63005	Phospholipase C gamma 1; substrate of many growth factor receptor and nonreceptor tyrosine kinases; produces second messenger molecules that are elements of signal transduction pathways related to cell proliferation.	Ubiquitous; hepatocytes; hepatic stellate cells; vascular smooth muscle; vascular endothelial cells
0.001	0.97	0.15	0.48	0.11	1.08	0.07	U58513	Rho kinase (p160, ROCK-2); Rho is a small GTPase; serine/threonine coiled coil-forming protein kinase; downstream targets include LIM-kinase 1, which phosphorylates cofilin, an actin-depolymerizing factor; regulates actin cytoskeletal	Ubiquitously expressed except in the brain and muscle

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								reorganization; Rho activity enhances secretion; phosphorylation of. myosin light chain and moesin may prevent pathologic platelet activation during atherogenesis.	
0.002	1.48	0.47	0.00	0.00	1.26	0.23	ET61257	Map kinase kinase kinase (MEKK 1); serine-threonine kinase; regulates sequential protein phosphorylation pathways involving mitogen-activated protein kinases (MAPKs), including some Jun kinases	
0.002	1.10	0.41	0.00	0.00	1.25	0.22	U85608 (was U11548)	Mitogen-activated protein kinase (MAPK); signal transduction; important in cell proliferation, differentiation, and apoptosis; induced by epidermal growth factor; activation of MAPK induces c-Fos and c-Jun; CR reduces the age related decline in MAPK activation	Liver (15 times higher in fetal than adult); ubiquitous
0.004	1.09	0.08	0.12	0.20	0.89	0.32	ET62570	Mad homologue Smad5; downstream component in the TGF-beta family signaling cascade, transduces signals from the	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								cell surface to the nucleus; participates in regulation of gene expression; essential in left/right isomerism and liver development; essential for angiogenesis	
0.001	1.24	0.31	0.00	0.00	1.18	0.27	D50095	Histamine H1 receptor; GTP-binding protein-coupled receptor; coupled to phosphoinositide turnover-calcium mobilization signaling pathway; regulates insulin-like growth factor I expression and cell proliferation; modulates IL-6 action; regulates physiological functions in neurons; regulates transport of thyroxine into hepatocytes	Liver, brain, spleen (ubiquitous)
0	1.09	0.16	0.00	0.00	1.15	0.13	U60330	Ki antigen (PA28 gamma); cell proliferation; the interferon-gamma (IFN-gamma)-inducible PA 28 activator complex enhances the generation of class I binding peptides by altering the cleavage pattern of the proteosome	Liver, neurons, broad tissue distribution
0.002	1.09	0.31	0.00	0.00	1.39	0.39	M31419	Interferon-activatable gene (204); mediates	Nucleoi

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								antimicrobial, immunomodulary and cell growth-regulatory activities of interferons; increased up to 75-fold by alpha-interferon treatment	
MEMBRANE RECEPTORS									
0.029	1.11	0.15	0.33	0.38	1.39	0.49	U40189	Pancreatic poly peptide/neuropeptide Y/peptide YY receptor (NPYR-D); G protein-coupled	Liver
0	1.00	0.15	0.02	0.03	1.15	0.10	U58367	Neuropeptide Y receptor Y5/Y6/Y2b (referred to as both Y5 and Y2b, has now been designated as Y6 in literature); (NPY-Y6); (neuropeptide Y is an important regulator of energy balance in mammals through its orexigenic, antithermogenic, and insulin secretagogue actions; expressed abundantly in the central nervous system); NPY receptors mediate a variety of physiological responses including feeding and vasoconstriction	Neurons, vascular smooth muscle cells
0.027	1.12	0.15	0.31	0.34	0.95	0.32	X76295	Melanocortin 5 receptor; G-protein-coupled receptor; stimulates adenylyl cyclase	Widely expressed

P	CR	std	CON	std	SW	std	GenBank	Description	Location
0.020	1.89	0.60	0.00	0.00	1.29	0.82	ET61559	Bradykinin 131 subtype receptor; G protein-coupled membrane bound; T-kininogen modulation during acute phase protein synthesis	Liver (ubiquitous)
0.013	2.14	0.64	0.17	0.16	1.38	0.69	X99581	Chemokine receptor; primary receptor stromal cell-derived factor/pre-B growth stimulating factor; seven transmembrane domain receptor	Liver and bone marrow
0.001	1.46	0.18	0.38	0.22	0.98	0.10	ET61693	Leptin receptor (OB-R); transmembrane receptor	Lung, liver, muscle, brain; developing bone, mesenchyme, notochord and liver (Am J Clin Nutr 1999 Jan; 69(1):18-21)
0.000	1.52	0.10	0.00	0.00	0.97	0.19	U28404	Macrophage inflammatory protein-1 alpha receptor; mediates growth inhibitory effects of the chemokine	MIP-1 alpha RL2 in liver and spleen
0.017	1.10	0.20	0.48	0.29	1.12	0.12	U57612	CD44; receptor for hyaluronan; cell surface glycoprotein; liver hyaluronan clearance from the blood; involved in lymphocyte homing and activation	Liver, central nervous system, lung, epidermis, and pancreas
<0.001	1.24	0.25	0.00	0.00	1.28	0.24	M86441	BEK fibroblast growth factor	Li +J520 liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								receptor (BEK FGF receptor, FGF-2), membrane-spanning tyrosine kinase; activated by three members of the FGF family; activation causes the foregut endoderm to develop into the liver	parenchymal cells and others
<0.001	1.04	0.05	0.00	0.00	1.13	0.20	U56734	Member of the macrophage mannose receptor type C (calcium dependent) lectin family; critical for processes ranging from cell adhesion to antigen presentation; gene family includes macrophage mannose, the phospholipase A2, and the DEC 205 receptors	Widespread tissue distribution; fetal liver; endothelialized sites; chondrocytes in cartilaginous regions of the embryo
0.002	1.17	0.15	0.06	0.11	1.27	0.38	X06368	Macrophage colony-stimulating factor-1 (CSF-1) receptor	Liver
0.001	1.13	0.15	0.02	0.03	1.21	0.34	X83933	Ryanodine receptor type 2; form Ca2+ channels in the membrane of the ER; intracellular calcium release channels controlling cytosolic calcium levels	ER; cardiac muscle; neurons; most excitable cells; liver
0.003	1.19	0.38	0.00	0.00	1.23	0.31	x57349	Transferrin receptor; cell surface glycoprotein; cell growth; binds the major serum iron-transport protein, transferrin, and mediates	Liver

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								cellular iron uptake	
<0.001	0.98	0.26	0.00	0.00	1.10	0.03	X84896	P2X purinergic receptor (P2XR) channels bind extraacellular ATP and mediate Ca(2 +) influx	Liver; ubiquitous
0	1.09	0.16	0.00	0.00	1.15	0.13	U60330	Ki antigen (PA28 gamma); cell proliferation; the interferon-gamma (IFN-gamma)-inducible PA 28 activator complex enhances the generation of class I binding peptides by altering the cleavage pattern of the proteosome	Liver, neurons, broad tissue distribution
CYTOSKELETON									
0.022	2.47	0.20	0.00	0.00	1.60	1.35	X05640	NF-M gene for middle-molecular-mass neurofilaments (like keratins)	Epithelial cells (mainly liver and brain)
0.006	1.07	0.19	0.40	0.23	1.09	0.10	ET62211	Formin; reorganization of the cytoskeleton, cytokinesis, stress fiber formation, and transcriptional activation of the serum response factor	Ubiquitous
0.033	1.42	0.83	0.00	0.00	1.41	0.51	X57377	Myosin heavy chain gene; novel and unique C-terminal region	CNS, cephalic ganglia, and spinal ganglia; lid skeletal myosin heavy chain gene expressed in fat-storing cells

P	CR	std	CON	std	SW	std	GenBank	Description	Location
									(FSC, lipocytes, or Ito cells) of regenerating liver
0.012	1.06	0.07	0.38	0.26	1.22	0.32	ET61336	Nonmuscle myosin heavy chain IIB; cell motility	CNS; lid skeletal myosin heavy chain gene expressed in fat-storing cells (FSC, lipocytes, or Ito cells) of regenerating liver
0.001	1.62	0.35	0.00	0.00	1.12	0.31	M91602	Myosin light chain 2; contractile protein	fat-storing cells (FSC, lipocytes, or Ito cells) of regenerating liver; muscle
0.000	1.90	0.15	0.00	0.00	0.97	0.35	V00830	Epidermal keratin subunit; intermediate filament protein; maintenance of epidermal cell shape and resistance to mechanical trauma	Liver (bile duct epthelium, epithelial cells)
0.001	0.97	0.15	0.48	0.11	1.08	0.07	U58513	Rho kinase (ROCK-2); (Rho is a small GTPase; serine/threonine protein kinase); Rho activity enhances secretion; phosphorylation of myosin light chain	Ubiquitously expressed except in the brain and muscle
0.038	0.97	0.47	0.49	0.02	1.32	0.20	ET61218	Microtubule-associated	Most cell types

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								protein 4 (MAP4); co-localizes with microtubules; expressed during developmental; likely involved in differentiation	including liver
TUMOR SUPRESSORS/ANTI-TUMOR FACTORS									
0.006	1.12	0.23	0.35	0.30	1.33	0.19	X97719	Friend-virus-susceptibility-1 gene (Fv1); prevents or delays spontaneous or experimentally induced viral tumors	
0.029	1.77	1.69	0.00	0.00	3.00	0.39	X74671	Neurofibromatosis type 2 gene; tumor supressor gene; cytoskeleton-membrane linker; mutant leads to CNS tumors	Ubiquitous
0	0.91	0.19	0.00	0.00	1.08	0.06	ET61211	RNA-dependent EIF-2 alpha kinase; double-stranded (ds) RNA-dependent protein kinase (PKR); key mediator of antiviral effects of interferon (IFN); active player in apoptosis	Ubiquitous
FUNCTION UNKNOWN									
0	1.23	0.24	0.00	0.00	1.13	0.16	X96737	Synaptobrevin-like gene (SYBL1); housekeeping gene; X-linked; inactivated on one X in every female cell, and also inactive on the Y of male cells	Ubiquitous
0.007	1.35	0.59	0.00	0.00	1.21	0.22	ET62791	WW domain binding protein 6; WW domain is a globular	Uncharacterized

P	CR	std	CON	std	SW	std	GenBank	Description	Location
								protein domain that is involved in mediating protein-protein interaction and that ultimately participates in various intracellular signaling events; WW domain mediates protein-protein interaction by binding proline-rich modules in ligands.	

APPENDIX B

Low-Hi-Low p value	CR-ave.	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
0.001	0.77	0.20	1.84	0.06	0.73	0.28	M95599	Homeobox-containing protein (Hox-1.11)	Most abundant in 12-day-old embryos and progressively decreases during further embryonic development.
0.001	0.54	0.04	1.85	0.27	0.91	0.28	X58196	Mouse H19 gene: The H19 gene produces an abundant developmentally regulated transcript of unknown function in normal embryos. It is subject to transcriptional regulation by parental imprinting, which results in the maternally inherited gene being expressed and the paternally inherited gene being repressed.	It is expressed at the blastocyst stage of development, and accumulates to high levels in tissues of endodermal and mesodermal origin. After birth the gene is expressed in all tissues except skeletal muscle. A muscle specific isoform has also been cloned
0.002	0.89	0.23	2.14	0.46	0.79	0.05	X99807	Selenoprotein P: covalently bound 8-12 selenocysteine residue. Its concentration is sensitive to the selenium status of the animal. Its function is unknown.	Liver, testis, brain, gut, and hematopoietic cells
0.005	1.17	1.26	4.28	0.80	0.75	0.16	J04953	Gelsolin: a Ca2+- and polyphosphoinositide 4,5-bisphosphate (PIP2)1-regulated actin filament severing and	Ubiquitous

Low-Hi-Low p value	CR-ave.	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
								capping protein that is implicated in actin remodeling in growing and in apoptotic cells	
0.044	0.68	0.78	1.96	0.53	0.33	0.58	L23971	Fragile X mental retardation syndrome protein (Fmr1) (mouse homologue): Fragile X Mental Retardation Syndrome is the most common form of hereditary mental retardation, and is caused by defects in the FMR1 gene. FMR1 is an RNA-binding protein and the syndrome results from lack of expression of FMR1 or expression of a mutant protein that is impaired in RNA binding. The specific function of FMR1 is not known	Brain
Not reported in liver, muscle, brain, blood:									
0.032	0.18	0.31	11.84	8.02	0.00	0.00	L02241	Mouse protein kinase inhibitor (testicular isoform): inhibitor protein of the cAMP-dependent protein kinase. This isoform of PKI is reported found only in testis	Testis specific
0.017	1.24	1.37	4.54	1.85	0.16	0.26	D89901	High-glycine tyrosine keratin type II.3	Hair
Blood, T and B cells									
0	0.77	0.12	2.89	0.34	1.23	0.42	X14061	Beta-globin complex DNA for y,	Blood

Low-Hi-Low p value	CR-ave.	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
								bh0, bh1, b1 and b2 genes, bh2 and bh3 pseudogenes:	
0.004	0.29	0.27	3.06	1.00	0.98	0.37	X53247	EN-7: [has 100% seq homology with RAS-related C3 botulinum substrate 2 (Rac2)]: a member of the ras gene superfamily. mRNA expression is restricted to the cells of hemopoietic lineages, mRNA levels increase with the terminal differentiation of hemopoietic cells into granulocytes.	T, B and myeloid hemopoietic cells
0.008	0.79	0.13	2.15	0.61	0.94	0.18	U09010	Mannose-binding protein A (Mbl1): a serum protein, a member of a family of collagenous lectins (collectins), that activates the complement system after binding to glycoconjugates found on the surface of microorganism	Blood
0.022	0.72	0.05	2.55	0.95	1.21	0.38	M22531	Mouse complement C1q B chain: Mouse complement component C1q is a serum glycoprotein which consists of six A chains, six B chains and six C chains.	Macrophages
<u>Energy Metabolism/Biosythesis</u>									
0.006	0.85	0.15	2.57	0.75	0.90	0.26	Y00309	Lactate dehydrogenase-A (LDH-A)	Liver, muscle

Low-Hi-Low p value	CR-ave.	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
0.018	0.97	0.15	2.87	1.18	0.73	0.24	X02520	Lactate dehydrogenase A4 isoenzyme	Liver, muscle
0.008	0.92	0.13	2.22	0.64	0.84	0.14	J05277	Hexokinase(HK): catalyzes the first step in glucose metabolism, that is, the conversion of glucose to glucose-6-phosphate (G6P)	Liver, muscle
0.047	0.92	0.07	2.10	0.88	0.79	0.33	X58426	Hepatic triglyceride lipase: an important enzyme that is involved in the metabolism of chylomicrons, intermediate density lipoproteins, and high density lipoproteins	Liver
0.001	0.00	0.00	43.66	12.59	1.17	1.77	U84207	CTP:phosphocholine cytidylyltransferase: Phosphatidylcholine (PC) is the most abundant eukaryotic phospholipids and serves critical structural and cell-signaling functions. CTP:phosphocholine cytidylyltransferase (CT) is the rate-limiting enzyme in the CDP-choline pathway of PC biosynthesis, which is utilized by all tissues and is the sole or major PC biosynthetic pathway in all non-hepatic cells	Ubiquitous
Extracellular Matrix									
0.009	0.83	0.17	2.32	0.67	1.02	0.22	M18194	Fibronectin (FN): an	Ubiquitous ?

Low-Hi-Low p value	CR-ave.	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
								extracellular matrix protein, is involved in the adhesion and migration of hematopoietic cells, found in many extracellular matrices as well as being abundant plasma proteins. The plasma isoforms of fibronectin, which are synthesized in the adult by liver hepatocytes, differ from those derived from most other cells and tissues due to alternative mRNA splicing	
Protein Turn-Over/Transport/Processing									
0	0.83	0.02	3.40	0.25	1.13	0.38	ET61037	Polyubiquitin: Ubiquitin is an omnipresent protein found in all eukaryotes so far analysed. It is involved in several important processes, including protein turnover, chromosome structure and stress response	Ubiquitous
0	0.73	0.12	2.11	0.33	0.98	0.06	X70303	Proteasome subunit MC3 (alpha type): The proteasome is a multisubunit 20 S proteinase complex involved in ubiquitin-dependent and -independent intracellular protein metabolism.	Ubiquitous?
0.001	0.00	0.00	16.01	3.18	2.78	3.98	D87899	ADP-ribosylation factor 2 (ARF2) ADP-ribosylation factors	Ubiquitous

Low-Hi-Low p value	CR-ave.	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
								(ARFs) are a family of small GTP-binding proteins that are involved in the formation of coated transport vesicles for protein secretion through the endoplasmic reticulum and Golgi vesicular trafficking system	
0.063	0.81	0.17	3.06	1.71	1.01	0.29	D78645	Glucose-regulated protein 78	Liver, adipose, brain, heart, kidney, lung, spleen, muscle, small intestine.
Signal Transduction									
0.004	1.50	0.32	2.48	0.57	0.83	0.09	M13964	Stimulatory G protein of adenylate cyclase, alpha chain: component signal transduction systems.	Ubiquitous
Transcription Factor									
0.037	0.94	0.10	2.14	0.85	0.82	0.24	X57638	Peroxisome proliferator activated receptor alpha: is activated by a diverse class of rodent hepatocarcinogens that causes proliferation of peroxisomes	Liver

APPENDIX C

Low-Low-Hi p value	CR-ave	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
0.013	5.61	9.71	5.11	4.87	40.81	18.89	X04591	Brain creatine kinase B: The creatine kinase-B (CKB) enzyme is proposed to have a pivotal role in the regeneration of ATP in the nervous system.	brain
0.013	1.48	1.77	1.09	0.94	4.52	1.34	M61705	Intestinal alkaline phosphatase (IAP); a membrane-bound metalloenzyme catalysing cleavage of inorganic phosphate nonspecifically from a wide variety of phosphate esters.	intestine, (kidney)
0.015	0.00	0.00	0.01	0.01	15.52	8.36	D78353	Eosinophil peroxidase; is one of the granule enzymes in the eosinophil-specific granules and is distinct from myeloperoxidase.	blood (eosinophils)
0.001	1.12	1.18	0.77	0.66	6.74	1.50	M12930	Erythropoietin; The glycoprotein hormone erythropoietin regulates the level of oxygen in the blood by modulating the number of circulating erythrocytes.	produced in the kidney or liver of adult and the liver of fetal or neonatal mammals
0	0.17	0.29	0.15	0.14	9.80	2.34	J05149	Insulin receptor (IF)	ubiquitous
0.009	0.00	0.00	0.00	0.01	7.52	3.82	U65586	Telomeric protein mTRF1; a telomere repeat binding factor packages the long tandem arrays of the double-stranded TTAGGG	ubiquitous

Low-Low-Hi p value	CR-ave	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
								sequence motif in mammalian telomeres.	
0.016	0.00	0.00	0.01	0.01	34.54	20.11	X14897	Fos B; a nuclear protein of 338 amino acids presenting a 70% homology with c-fos, whose expression is activated during G0/G1 transition. Similar to c-fos, fos B protein plays a role in control of gene expression.	ubiquitous
0.005	0.98	0.98	0.66	0.56	3.62	1.12	X66225	Retinoid X receptor-gamma (mRXR- gamma); a kind of nuclear receptors of retinoids which play a fundamental role in regulating normal cell proliferation and differentiation. The retinoid X receptors (RXRs) regulate gene expression by forming transcriptionally active heterodimeric RAR (the retinoic acid receptors)/RXR or homodimeric RXR/RXR complexes on DNA.	ubiquitous (one isoform in adrentals, kidney, and liver; another in brain and lungs; both are expressed strongly in heart and muscle)
0.023	2.31	3.18	1.84	1.63	16.67	9.38	X54239	Evx1 protein; A murine even-skipped (eve) homologue. During embryogenesis, Evx 1 shows a biphasic expression pattern. The early and late transcription pattern is compatible with a role of Evx 1	embryos

Low-Low-Hi p value	CR-ave	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
								in specifying posterior positional information along the embryonic axis and in specifying neuronal cell fates within the differentiating neural tube.	
0.006	0.00	0.00	0.00	0.00	55.35	24.84	X70800	Wnt-11 protein; The Wnt gene family encodes a set of signalling molecules, thought to play an important role in key processes of embryonic development. WNT11 has possible roles in the development of skeleton, kidney and lung.	embryos (truncus arteriosus, somites at the medial junction of the dermatome and the myotome, and limb bud mesenchyme)
0.039	1.10	0.18	0.44	0.57	4.73	3.06	X99796	Tsx; a gene of unknown function that was shown to be expressed specifically in the testis. It locates 3' form the Xist gene which involves in the X inactivation.	tetis
0.043	0.00	0.00	0.01	0.02	15.23	11.16	X14770	Rds protein/peripherin; a photoreceptor disc membrane-associated glycoprotein involved in retinal degeneration slow. It is 92.5% identical to the sequence of the bovine photoreceptor-cell protein peripherin. It may function as an adhesion molecule for stabilization of the outer segment discs.	eye?

Low-Low-Hi p value	CR-ave	std	Cont-ave	std	Sw-ave	std	GenBank	Name/Description	Tissue
0	0.99	1.04	0.68	0.59	15.29	2.39	X57302	Hepatitis virus MHV-A59 defective interfering (DI) RNA; RNA of defective-interfering virus formed earlier in infection, can mediate homologous interference.	?

APPENDIX D

Hi-Hi-Low p value	CR-ave	std	Cont-ave	std	Switched -ave	std	GenBank	Name/Description	Tissue
0	1.42	0.20	1.17	0.30	0.04	0.07	AF009414	SOX11; Sox genes, which encode transcription factors related by a DNA-binding motif termed the HMG box, are known to have diverse roles in vertebrate differentiation and development. SOX11 was suggested a role in neuronal maturation and an additional role in tissue modelling during development.	ubiquitous
0.041	1.01	0.43	1.21	0.25	0.38	0.23	L40156	surfactant protein D (Sftp4); Surfactant protein-D (SP-D) is a collectin found associated with surfactant in the lung. SP-D has also been functionally characterized as an opsonin for diverse microorganisms and a chemoattractant for phagocytic cells.	predominantly in lung, and also in heart, stomach, and kidney but not in brain
0.013	1.02	0.10	1.26	0.17	0.54	0.29	D86176	Phosphatidylinositol 4-phosphate 5-kinase-alpha; the type I phosphatidylinositol-4-phosphate 5-kinase (PI4P5K) have been identified as one of the cytosolic components required for ATP-dependent, Ca2+-activated secretion.	highly expressed in the brain and testis, but barely detectable in the liver and skeletal muscle
0.032	1.16	0.33	1.31	0.27	0.61	0.07	Z36293	Sialoadhesin; Sialoadhesin is a macrophage-restricted adhesion	expressed strongly by macrophages in

Hi-Hi-Low p value	CR-ave	std	Cont-ave	std	Switched -ave	std	GenBank	Name/Description	Tissue
								molecule of 185 kDa that mediates sialic acid-dependent binding to cells.	lymphoid and haemopoietic tissues

APPENDIX E

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
0.001	3.65	0.92	0.32	0.10	1.10	0.33	D83262	Neuronal glutamate transporter EAAT4: induces high-affinity uptake of L-glutamate that is dependent on external Na + .	Brain (neurons)
0.014	2.73	0.31	0.63	1.09	0.42	0.52	X90778	Histone H2B	testis specific
0.017	2.25	0.34	0.79	0.74	0.51	0.50	M96760	rod outer segment membrane protein 1 (Rom1): Rom-1 and peripherin are related retina-specific integral membrane protein localized to the photoreceptor disk rim, where they may act jointly in the photoreceptor disk biogenesis.	eye?
0.018	7.93	4.23	0.00	0.00	1.16	1.25	X14971	alpha-adaptin (A): Adaptins are components of the adaptor complexes which link clathrin to receptors in coated vesicles. The alpha-adaptins, which are found exclusively in endocytic coated vesicles	brain and liver
0.02	7.76	4.74	0.00	0.00	0.00	0.00	D49429	PW29: calcium binding protein with oligoproline motif, a mouse homolog of Mcd1 pS.c./Rad21 S.p., has been implicated functioning in sister chromatid cohesion.	strongly expressed in the testis, brain, kidney and heart
0.022	10.70	6.08	0.00	0.00	1.38	1.61	M55617	mast cell protease-4: a	connective tissue

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								secretory granule serine protease of the peritoneal connective tissue mast cells (CTMC).	
0.023	1.78	0.51	0.84	0.63	0.21	0.31	AF013253	preprocortistatin (Cort): Cortistatin is a 14- residue putative neuropeptide with strong structural similarity to somatostatin and is expressed predominantly in cortical GABAergic interneurons. Administration of cortistatin into the brain ventricles specifically enhances slow-wave sleep, presumably by antagonizing the effects of acetylcholine on cortical excitability.	brain (cerebra cortex and hippocampus)
0.032	2.12	0.81	0.73	0.61	0.37	0.39	U02982	secretogranin III (SgIII): an acidic chromogranin/secretogranin-like protein of unknown function that is present in the storage vesicles of many neuroendocrine cells.	brain- and pituitary-specific
0.033	1.70	0.28	0.70	0.52	0.54	0.45	U39818	tuberin (TSC2): the tuberous sclerosis 2 (TSC2) gene product, which contains an activity that specifically	ubiquitous

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								stimulates the intrinsic GTPase activity of Rapt a and may acts as a presumed tumor-suppressor.	
0.037	21.18	14.98	0.00	0.00	0.00	0.00	D17407	U2af1-rs1(SP2); encodes a protein with significant similarity to U2 small nuclear ribonucleoprotein auxiliary factor small subunits, an essential mammalian splicing factor; an endogenous imprinted gene on the proximal region of chromosome 11. This gene is transcribed exclusively from the unmethylated paternal allele, while the methylated maternal allele is silent.	Ubiquitous
0.038	16.26	11.58	0.00	0.00	0.00	0.00	X72862	Beta-3-adrenergic receptor; a member of the super-family of G protein-coupled receptors; plays a role in the control of cAMP accumulation and may be involved in the control of energy expenditure in fat tissue.	mainly expressed in mouse brown and white adipose tissues
				Transcription Factor					
0.007	2.57	0.46	0.70	0.71	0.41	0.52	L10409	Fork head. related protein	Adult live,

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								(HNF-3 beta): in addition to its known functions as transcriptional activators in adult liver, play a role in , body axis formation, neural tube patterning and definitive endoderm formation during gastrulation.	embyonic node, notochord, floor plate and gut
0.007	36.94	12.40	0.00	0.00	6.27	10.87	X86368	Transcription factor FKH-2: a member of the "winged helix" or "forkhead" transcription factor family; expression patterns of the fkh-2 gene and HNF-3 beta, are overlapping in early stages of gestation.	Expressed in embryos and becomes restricted to the midbrain
0	12.24	3.07	0.00	0.00	0.80	1.39	X61754	Heat shock transcription factor 2; binds to the heat shock element (HSE).	Ubiquitous
0.001	4.43	0.96	0.00	0.00	1.05	0.77	L77247	Zinc finger protein (kid-1); a putative renal transcription factor; regulation during ontogeny and in response to ischemia and toxic injury	Kidney and eye
0.024	16.08	10.27	0.00	0.00	0.00	0.00	X92592	Fkh-5 (also known as Mf3 and TWH); a member of the 'winged helix' or 'forkhead' transcription factor gene family	Specifically expressed in the developing central nervous system
0.007	36.92	17.87	0.13	0.22	0.00	0.00	U66620	SWI/SNF complex 60 KDa subunit (BAF60a);	Ubiquitous

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								mammalian homologue of yeast SWI/SNF complex; also referred to as BRG 1-associated factors (BAFs); facilitates the function of transcriptional activators by opposing chromatin-dependent repression of transcription, and (in mamals) is likely dedicated to developmentally distinct functions.	
0.005	1.95	0.59	0.87	0.26	0.22	0.17	U83148	NFIL3/E4BP4 transcription factor; nuclear factor regulated by IL-3/adenovirus E4 promoter binding protein in a distinct growth factor-regulated signaling pathway that is responsible for the survival of early B-cell progenitors	Blood
					Cell Growth/Cycle				
0	14.42	3.83	0.00	0.00	0.00	0.00	L25602	Bone morphogenetic protein 2 (BMP-2); pleiotropic functions range from extraskeletal and skeletal organogenesis to bone generation and regeneration; structurally related to	Ubiquitous

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								transforming growth facto-beta s, activins, and inhibins	
0.004	2.97	0.78	0.34	0.60	0.33	0.58	D89080	Fibroblast growth factor 10 (FGF10): has important roles in mediating mesenchymal-epithelial cell interactions during embryogenesis. In particular, Fgf10 is predicted to function as a regulator of brain, lung and limb development, prostatic growth and development and so on.	expressed relatively abundantly if, embryos and the lung, and at much lower levels in brain and heart
0.002	14.09	4.67	1.12	1.93	0.00	0.00	M30903	B lymphocyte kinase (blk); a Src family tyrosine kinase specific to B lymphoid cells	Blood (specifically expressed in the B cell lineage)
0.007	4.14	1.40	0.00	0.00	1.09	1.14	X59398	Tyrosine kinase receptor of the PDGFR/CSF1 R family (Flt-3); involved in development and function of various cell lineages; unidentified ligand in placenta, gonads and hematopoietic and nervous systems.	various adult tissues including gonads and brain, and in hematopoietic cells
0.008	1.64	0.34	0.00	0.00	0.71	0.62	U22399	Cdk-inhibitor p57KIP2 (KIP2); a potent, tight-binding inhibitor of several G1 cyclin/Cdk complexes; and is suggested to be involved in	High level expression in skeletal muscle, brain, heart, lungs, and eye

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								decisions to exit the cell cycle during development and differentiation.	
					Extracellular Matrix				
0.023	24.61	5.47	0.00	0.00	7.30	12.64	M32136	alpha-1 type IX collagen (COL9A1): a structural component of the extracellular matrix of connective tissues	ubiquitous
0.035	2.93	1.06	0.64	1.10	0.81	0.21	U43541	s-laminin (also called laminin beta 2): a homologue of the 131 (beta 1) chain of the widely distributed basal lamina (BL) glycoprotein, laminin. It may affect postsynaptic differentiation.	muscle
					DNA respair				
0.039	14.29	8.65	0.00	0.00	2.67	3.79	L26320	FLap endonuclease-1 (FEN-1): an enzyrne which functions in double-strand break repair flap resolution; it specifically cleaves DNA flap strands that terminate with a 5' single-stranded end; in addition to endonuclease activity, FEN-1 has a 5'-3' exonuclease activity which is specific for double-stranded	Ubiquitous

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								DNA.	
0.005	0.46	0.08	1.07	0.27	1.22	0.14	L11333	Mouse carboxyesterase; serine-dependent enzymes	Predominantly in male livers
0.049	0.51	0.45	1.18	0.06	1.06	0.14	M74149	Creatine kinase B; plays an important role in buffering ATP and ADP levels in tissues which have intermittently high and fluctuating energy demands	liver, brain, skeletal muscle, heart, intestines
0.014	0.56	0.06	1.16	0.25	1.27	0.27	M17122	Complement 4b-binding protein (C4b-binding protein);an abundant oligomeric plasma glycoprotein which controls the activation of the complement cascade through the classical pathway .	Liver
0.02	0.30	0.39	1.16	0.32	1.38	0.34	U36393	TFEB; a member of the microphthalmia-TFE (MiT) subfamily of basic helix-loop-helix leucine zipper (bHLH-ZIP) transcription factors.	Liver, brain, skeletal muscle, heart, spleen, lung, kidney, testis
0.025	0.58	0.11	1.09	0.31	1.28	0.25	D70849	Zic3; encodes a zinc finger protein, is expressed in the developing or matured central nervous system in a highly restricted manner. It's the vertebrate homologue of Drosophila odd-paired, which may play an essential role in	Restricted in the cerebellum at the adult stage.

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
								parasegmental subdivision and in visceral mesoderm development.	
0.046	0.50	0.21	1.37	0.35	1.49	0.57	D37837	65-kDa macrophage cytosolic protein; is phosphorylated specifically in LPS-stimulated murine macrophages. a murine homologue of human L-plastin, recently identified as a novel transformation-induced polypeptide of neoplastic human cells. Its function is implicated in macrophage activation by LPS.	Hemopoitetic cells
0.01	0.27	0.13	1.55	0.51	1.39	0.35	U04268	Mouse stem cell antigen Sca-2 precursor; a member of the Ly-6 family, a group of small cysteine-rich cell surface proteins that are anchored in the membrane by a glycosyl-phosphatidylinositol moiety.	Early thymic precursor and mature peripheral B cells (not mature thymocytes and peripheral T cells)
0.018	0.51	0.05	1.20	0.24	1.37	0.40	D38580	Vomeronasal secretory protein I (VNSP I);secretory protein, member of the lipocalin superfamily	Specifically expressed in vomeronasal and posterior glands of the nasal septum, the ducts of which open into the lumen of

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
									the vomeronasal organ
0.024	0.27	0.08	1.03	0.07	1.48	0.67	M27501	Protamine 2; the predominant nuclear proteins of mammalian spermatozoa, is regulated during germ cell development	Testis-specific

APPENDIX F

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched -ave	std	GenBank	Name/Description	Tissue
0.005	0.46	0.08	1.07	0.27	1.22	0.14	L11333	Mouse carboxyesterase; serine-dependent enzymes	Predominantly in male livers
0.049	0.51	0.45	1.18	0.06	1.06	0.14	M74149	Creatine kinase B; plays an important role in buffering ATP and ADP levels in tissues which have intermittently high and fluctuating energy demands	liver, brain, skeletal muscle, heart, intestines
0.014	0.56	0.06	1.16	0.25	1.27	0.27	M17122	Complement 4b-binding protein (C4b-binding protein);an abundant oligomeric plasma glycoprotein which controls the activation of the complement cascade through the classical pathway .	Liver
0.02	0.30	0.39	1.16	0.32	1.38	0.34	U36393	TFEB; a member of the microphthalmia-TFE (MiT) subfamily of basic helix-loop-helix leucine zipper (bHLH-ZIP) transcription factors.	Liver, brain, skeletal muscle, heart, spleen, lung, kidney, testis
0.025	0.58	0.11	1.09	0.31	1.28	0.25	D70849	Zic3; encodes a zinc finger protein, is expressed in the developing or matured central nervous system in a highly restricted manner. It's the vertebrate homologue of Drosophila odd-paired, which may play an essential role in parasegmental subdivision and in visceral mesoderm development.	Restricted in the cerebellum at the adult stage.

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched-ave	std	GenBank	Name/Description	Tissue
0.046	0.50	0.21	1.37	0.35	1.49	0.57	D37837	65-kDa macrophage cytosolic protein; is phosphorylated specifically in LPS-stimulated murine macrophages. a murine homologue of human L-plastin, recently identified as a novel transformation-induced polypeptide of neoplastic human cells. Its function is implicated in macrophage activation by LPS.	Hemopoitetic cells
0.01	0.27	0.13	1.55	0.51	1.39	0.35	U04268	Mouse stem cell antigen Sca-2 precursor; a member of the Ly-6 family, a group of small cysteine-rich cell surface proteins that are anchored in the membrane by a glycosyl-phosphatidylinositol moiety.	Early thymic precursor and mature peripheral B cells (not mature thymocytes and peripheral T cells)

Hi-Low-Low p value	CR-ave	std	CONT-ave	std	Switched -ave	std	GenBank	Name/Description	Tissue
0.018	0.51	0.05	1.20	0.24	1.37	0.40	D38580	Vomeronasal secretory protein I (VNSP I);secretory protein, member of the lipocalin superfamily	Specifically expressed in vomeronasal and posterior glands of the nasal septum, the ducts of which open into the lumen of the vomeronasal organ
0.024	0.27	0.08	1.03	0.07	1.48	0.67	M27501	Protamine 2; the predominant nuclear proteins of mammalian spermatozoa, is regulated during germ cell development	Testis-specific

APPENDIX G

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
Transcription Factor/Nuclear Receptor		
Y00850	Zinc finger protein 2 (Zfp2); Mkr-2; differentiation and/or maintenance of neurons	Brain (Central and peripheral neurons)
X63963	Paired box protein (Pax-6); transcription factor	Developing CNS
X06762	Homeo box B7 (Hoxb7); transcription factor; embryonic development; haematopoiesis	Developing embryo; blood; bone marrow cells; natural killer cells
X74040	Homeo box A9 (Hoxa9); transcription factor	Embryogenesis
X59251	Homeo box msh-like 1 (Msx1); transcription factor; early stage of eye developmental regulation in embryo	Embryogenesis
Z67747	Zinc finger protein 62 (Zfp62); a member of a multigene family encoding Zn mediated nucleic acid binding proteins	Embryonic development and Skeletal, cardiac muscle, and spleen in adult
M36516	Zinc finger protein 28 (Zfp28): a member of a multigene family encoding Zn mediated nucleic acid binding proteins	Embryonic development, testes in adult
U48721	Zinc finger protein 60 (Zfp60); a member of a multigene family encoding Zn mediated nucleic acid binding proteins; Kruppel associated boxes; associated with transcriptional control	Expressed transiently during muscle differentiation
X04435	Glucocorticoid receptor 1 (Grl1); energy balance; substrate uptake; liver	Liver
X74134	Nuclear receptor subfamily 2, group F member 1 (Nr2f1); COUP-TF1; orphan steroid hormone receptor; transcription factor	Liver
D00925	Transcription elongation factor A 1(Tcea 1); transcription	Liver

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	elongation factor	
X89264	Zinc finger protein 37 (Zfp37); putative transcription factor; peroxisome proliferator responsive	Liver
X56182	Myogen factor 5 (Myf5); transcription factor	Liver and heart (embronic)
X76653	Nuclear receptor subfamily 2, group F member 2 (Nr2f2); apolipoprotein regulatory protein 1; member of the COUP-family of steroid hormone orphan receptors	Liver, lung, kidney
L24118	Tumor necrosis factor induced protein 2 (Tnfip2): putative transcription factor	Liver; monocytes
U36575	Nuclear factor of activated T cells, cytoplasmic 2 (Nfatc2); T cell transcription factor isoform	Lymphocytes
U19463	Tumor necrosis factor induced protein 3 (Tnfip3); putative helix-loop-helix transcription factor activated in T-cell acute lymphoblastic leukemia	Lymphocytes
U19463	Tumor necrosis factor induced protein 3 (Tnfip3); putative helix-loop-helix transcription factor activated in T-cell acute lymphoblastic leukemia	Lymphocytes
ET61028	Sine oculis-related homeobox 1 homologue (Drosophila) (*Six 1*); AREC3	Many cell-types during development
U13878	RE1-silencing transcription factor (Rest); transcription factor; represses expression of neuronal genes	Many nonneuronal cells and tissues
Y12293	Forkhead box F2 (Foxf2); transcription factor; a developmental regulator in embryonic development	Mesodermal tissues and embroyonic: central nervous system, eye, ear, and limb bud
X60034	Homeo box D1 (Hoxd1); transcription factor; neurogenesis	Neurogenesis
ET63177	Pax-4 (Pax4); a paired-box transcription factor that plays an important role in the development of pancreatic beta/delta	Pancreatic islet endocrine progenitor cells

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	cells; role in endocrine cell development	
M81077	T-cell acute lymphocytic leukemia 2 (Tal2); putative basic helix-loop-helix transcription factor activated in T-cell acute lymphoblastic leukemia	T cells
X72697	Meiosis-specific XMR (Xmr); transcriptional activator function?	Testis; lymphoid cell lineages; nuclei of spermatocytes, early in the prophase of the first meiotic division, arid later becomes concentrated in the XY nuclear subregion
X76858	E4F transcription factor 1 (E4f1); DNA binding transcription factor	Ubiquitous
X15842	Reticuloendotheliosis (Rel); c-rel: member of the Rel/nuclear factor (NF)-kappaB family of transcriptional factors	Ubiquitous
X60136	Trans-acting transcription factor 1 (Sp1); transcription factor; component of some hepatic glucose response elements	Ubiquitous
X80508	Yes-associated protein, 65 kDa (Yap); transcription activator	Ubiquitous
ET61461	G-protein coupled receptor; poorly characterized	Unknown
<u>Translation/Splicing/RNA Processing Factors</u>		
Y08260	Cytoplasmic polyadenylation element binding protein (Cpeb); RNA binding protein that promotes polyadenylation and translational activation	Ubiquitous
X91656	Splicing factor arginine/serine-rich 3 (Sfrs3); splicing factor belonging to the highly conserved family of SR proteins; regulation of constitutive and alternative splicing	Ubiquitous
U28419	Translation initiation factor eif-4C homologue	Ubiquitous

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
Signal Transduction/Cell Cycle and Growth		
L28756	Gonadotropin releasing hormone receptor (Gnrhr); G-protein-coupled receptor; activates MAPK cascades	Brain (anterior pituitar), reproductive organs
Z31663	Activin A receptor, type 1B (Acvr1b); serine/threonine kinase receptor; a downstream transducer of activin signals	Brain (cerebral cortex, olfactory tubercle, and hippocampus)
X66118	Glutamate receptor, ionotropic, kainate 1 (Grik1)	Brain (CNS)
L41495	Proviral integration site (Pim2); serine/threonine kinase 2; cell proliferation; mitogen stimulated; long-term potentiation in hippocampus	Brain (CNS), Immune and epithelial cells
Z72000	B-cell translocation gene 3 (Btg3); negative control of cell cycle	Brain, fibroblast
X79082	Eph receptor A7 (Epa7); developmental kinase 1; member of receptor tyrosine kinase family	Brain, testes and spleen
Z27088	Relaxin (Rln); insulin gene family; remodeling of collagen	Brain, uterus, prostate gland, pancreas and kidney
X58287	Protein tyrosine phosphatase, receptor-type, M (Ptprm)	Capillaries in developing neural tissue, lung
ET61628	Phosphatidylinositol 3-kinase regulatory subunit, polypeptide 1 (p85alpha) (Pik3r1); role in cell growth, differentiation, survival, and vesicular transport	Liver
V00829	kallikrein 6 (Klk6); a member of multigene subfamily of serine protease that act on a diverse number of substrates, including several growth factors and extracellular matrix glycoproteins and proteinases;	Liver
Z22821	Rab23; Ras-related small GTPase; protein trafficking; central regulatory elements of the intracellular transport machinery; regulate vesicle docking and fusion, organelle dynamics	Liver

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
M25513	Guanine nucleotide binding protein, alpha transducing 1 (Gnat1)	Liver and others
M63658	Guanine nucleotide binding protein beta 4 (Gnb4)	liver, brain, blood cell
U38501	Guanine nucleotide binding protein, alpha inhibiting 1 (Gnai1)	Liver; cerebral cortex; pancreatic acinar cells; white adipose tissue; others
D30743	Wee1 homologue (S. pombe) (Wee1); inhibits entry into mitosis by phosphorylation of the Cdc2 kinase	Lymphocytes
ET61263	Spleen protein kinase (Syk); signal transduction	Lymphopoiesis; haematopoietic cells, platelets, macrophages and neutrophils
Z48757	Intestinal tyrosine kinase; protein tyrosine kinase	Mammary gland and intestine
ET61665	Discs-large tumor suppressor homologue (dlgh1); important role in the localization and function of glutamate receptors and K(+) channels	Neurons; epithelial cells
ET61399	G protein alpha olfactory subunit; sensory transduction	Olfactory epithelium
M14537	Acetylcholine receptor beta (Acrb)	Skeletal muscle
X92523	Calpain 3 (Capn3); intracellular calcium-dependant cysteine proteinase; tissue specific myofibrogenesis, modifies ryanodine receptor Ca2+ release channel	Skeletal muscle
Z11574	Son of sevenless 1, homologue 1 (Drosophila) (Sos1); Ras-specific exchange factor	T cells
Z11664	Son of sevenless 2 homologue 2 (Drosophila) (Sos2); Ras-specific exchange factor	T cells
U10440	Cyclin-dependent kinase inhibitor 1B (P27) (Cdkn1b); cell cycle	Ubiquitous
ET61257	MAP kinase kinase kinase (Map3k1); serine-threonine kinase;	Ubiquitous

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	regulates sequential protein phosphorylation pathways involving mitogen-activated protein kinases (MAPKs)	
S45828	NIMA-related expressed kinase (Nek1)	Ubiquitous
U65313	Ras-GTPase-activating protein SH3-domain binding protein 2(G3bp2-pending); essential for Ras signaling;	Ubiquitous
ET62740	Ankyrin 3 (Ank3); implicated in Na(+) channel clustering and activity; neuronal axons	Wide distribution
Hormone/Growth Factor/Cytokine/Chemokine		
X07962	Interleukin 7 (IL-7); growth factor	B cell progenitors
U66201	Fibroblast growth factor homologous factor 1 (FGF-1); nervous system development and function	Brain, skeletal muscle and other
U66204	Fibroblast growth factor homologous factor 4 (FHF-4) involved in nervous system development and function	Brain (CNS)
X99572	C-fos-induced growth factor (FIGF); secreted dimeric protein member of the platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF) family; mitogenic and morphogenic activity on fibroblasts	Endothelial cells, expressed in many tissues (including liver) during embryonic development
J00424	Interferon-beta	Liver
X07751	Thyroid hormone receptors	Liver
ET62118	Keratinocyte growth factor/fibroblast growth factor-7 precursor (mKGF)	Liver epithelial cells
X57413	Transforming growth factor-beta2 (TGFbeta2); cell proliferation	Liver stellate cells
ET62976	Macrophage inflammatory protein receptor 1-alpha 2; Induces	Liver, brain, thymus, heart, spleen

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	mobilization of intercellular calcium; beta-chemokine; leucocyte chemoattractant	
X53798	Small inducible cytokine subfamily, member 2 (Scyb2)	Macrophages
V00428	Lysozyme; signaling molecule for mast cells which respond with histamine secretion	Macrophages, paneth cells (located in duodenal crypts)
ET61471	Mast cell protease 7 (mMCP-7); mouse mast cell tryptase 2; released when mast cells are activated	Mast cells
U28404	Macrophage inflammatory protein-1 alpha receptor; mediates growth inhibitory effects of the chemokine	MIP-1 alpha RL2 in liver and spleen
U58367	Neuropeptide Y receptor Y5/Y6/Y2b (referred to as both Y5 and Y2b, has now been designated as Y6 in literature); (NPY-Y6); neuropeptide Y is an important regulator of energy balance in mammals through its orexigenic, antithermogenic, and insulin secretago	Neurons, vascular smooth muscle cells
U10092	Killer cell lectin-like receptor, subfamily A, member 6 (KIra6); Ly-49F; NK cell surface antigen; determinant of IL-2-activated NK cell specificity; inhibitory receptor for interaction with MHC class I proteins	NK cells
M31419	Interferon-activatable, gene (204); mediates antimicrobial, immunomodulary and cell growth-regulatory activities of interferons; increased up to 75-fold by alpha-interferon treatment	Nucleoi
X04725	Preproinsulin gene I	Pancreas and islets
X04724	Preproinsulin gene II	Pancreas and islets
M92416	Fibroblast growth factor (Fgf6); Fgf6 is the only known member of the FGF family whose expression is restricted to	Skeletal muscle

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	the muscle cell lineage during development	
X58995	Calmodulin-dependent protein kinase IV; multifunctional, serine-threonine protein kinase	T cells
V00756	Interferon beta (type 2)	T cells
M26271	Interleukin 2 receptor; cytokine receptor	T cells
D13695	Lymphocyte antigen 84 (Ly84); signal transduction protein 2	T cells
M28587	Alpha leukocyte interferon (MuIFN-alpha A); inhibition of cell proliferation	Ubiquitous
U49866	killer cell lectin-like receptor, subfamily A, member 3 (Klra3); interact with MHC class I (MHC-I) molecules on target cells	natural killer cell
DNA Replication/Repair/Apoptosis		
L31532	Bcl-2-beta; suppresses programmed cell death	Liver
Z37110	Cyclin G; augments apoptosis; target gene of P53	Liver
U25691	Lymphocyte specific helicase; putative role in replication, repair, recombination and transcription	T and B cells
L15435	Tumor necrosis factor (ligand) superfamily, member 9 (Tnfsf9),a member of the TNF family; proapoptosis facotor	T cells
ET62746	Brca2 gene; familial breast cancer susceptibility gene; important in DNA double-strand break repair (DSBR) and DNA damage-induced cell-cycle checkpoint activation	Ubiquitous
U04269	Caspase 1 (Casp1); cysteine protease mediator of apoptosis	Ubiquitous
X58472	KIN17, DNA-binding, nuclear protein, upregulated in response to UV and ionizing radiation; accumulated in the	Ubiquitous

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	nucleus of proliferating fibroblasts; overexpression inhibits progression into S phase	
ET63479	MLH1; DNA mismatch repair gene; function in mutation avoidance; cell cycle checkpoint control; cytotoxicity of various DNA-damaging agents; transcription-coupled nucleotide excision repair	Ubiquitous
ET61211	RNA-dependent EIF-2 alpha kinase; double-stranded (ds) RNA-dependent protein kinase (PKR); key mediator of antiviral effects of interferon (IFN); active player in apoptosis	Ubiquitous
X74351	XPAC (Xeroderma Pigmentosum group A Correcting protein); nucleotide excision DNA repair	Ubiquitous
X71978	Ft1, a novel gene related to ubiquitin-conjugating enzymes; deletion leads to partial syndactyly of the limbs and thymic hyperplasia, suggesting impaired programmed cell death	
Transporter/Channel/Pumps		
Y09108	sodium channel, type X, alpha polypeptide (Scn10a); ion channel; small-diameter sensory neurons associated with unmyelinated axons express a tetrodotoxin-insensitive (TTXi) voltage-gated sodium channel (VGSC); may play an important role in the transmission of nociceptive information	Brain
U14420	gamma-aminobutyric acid (GAGA-A) receptor, subunit beta 3 (Gabrb3); link binding of GABA (gamma-aminobutyric acid) to inhibitory chloride flux	Brain (CNS)
U48397	Mercurial-insensitive water channel 1 (mMIWC1); allows water and small solutes to pass	Brain, eye, lung, kidney, heart, muscle
X97281	K + channel beta-subunit, ion channel	Brain, Kidney

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
ET61590	Putative capacitative calcium entry channel (Trp6); involved in calcium entry secondary to activation of receptors coupled by the Gq class of G protein	Brain, kidney, heart and lung
X63100	Gap junction membrane channel protein alpha 7 (Gja7); connexin45; gap junction protein; ion exchange channel	Brain, lung, brain, heart, intestine, kidney
ET63385	Gap junction membrane channel protein beta 6 (Gjb6); connexin 30; forms transmembranous gap junction channels between adjacent cells	Brain; skin
L42340	Sodium channel 27	Brain; tissue distribution and protein poorly characterized
ET61440	Trp-related protein 3; cation channel; essential for agonist-activated capacitative Ca2+ entry; putative subunits of CCE channels	Endothelium
M23383	Glucose transporter 2	Liver
D29797	Syntaxin 3A, IER vesicular transport, membrane fusion	Liver
X83933	Ryanodine receptor type 2; form Ca2+ channels in the membrane of the ER; intracellular calcium release channels controlling cytosolic calcium levels.	Liver, neuron, cardiac muscle
ET62883	Skeletal muscle chloride channel	Skeletal muscle
X80417	MB-IRK2 (second class of inward rectifier potassium channels); ion channel	Skeletal muscle, heart, kidney
M30440	Potassium channel gene (MK2); shaker subfamily	T cells; myelinating Schwann cells
U03723	AKR voltage-gated potassium-channel (KCNA4)	Ubiquitous
U49393	ATPase (Atp2a3); Ca+ + transporting, ion pupmp	Ubiquitous
X84896	Purinergic receptor P2X, ligand-gated ion channel 1 (P2rx1);	Ubiquitous

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	mediate Ca(2+) influx; liver, ubiquitous	
ET63248	RAN binding protein 1 (RANBP1); RAN-specific GTPase-activating protein; required for nucleocytoplasmic transport of many types of cargo	Ubiquitous
U19521	Vesicle transport protein (munc-18c)	Ubiquitous
Chromatic Structure		
J03482	Histone H1; chromatin structure	Ubiquitous
ET62262	Histone H1b; chromatin structure	Ubiquitous
X16495	Histone H2A; chromatin structure	Ubiquitous
ET62908	Histone H2B; chromatin structure	Ubiquitous
U62672	Histone H3.1-D (H3-D) and histone H4-D (H4-D); chromatin structure	Ubiquitous
U62675	Histone H3.2-616, and histone H2b-616; chromatin structure	Ubiquitous
U62669	Histone H3.2-F (H3-F), histone H2a.1-F (H2a-F), histone H2b-F (H2b-F); chromatin structure	Ubiquitous
Biosynthesis and Metabolism		
X92122	UDP-glucuronosyltransferase 8 (Ugt8); key enzyme in cerebroside and sulfatide biosynthesis; glycosphingolipids; most abundant in myelin	Brain (CNS and PNS); tissue distribution poorly characterized
Y12257	Glutamic acid decarboxylase 67 kD; a rate-limiting enzyme in the biosynthesis of the neurotransmitter, gamma-aminobutyric acid (GABA)	Brain (CNS)
D49438	25-hydroxyvitamin D3 24-hydroxylase; metabolism and	Kidney and intestine.

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	regulation of vitamin D3	
X07888	3-hydroxy-3-methylglutaryl coenzyme A reductase; key regulatory enzyme for cholesterol biosynthesis.	Liver
D21826	Cytidine monophospho-N-acetylneuraminic acid hydroxylase; the key enzyme for the synthesis of N-glycolylneuraminic acid (NeuGc)	Liver
U00932	Glutamine fructose-6-phosphate transaminase 1 (Gfpt1); rate-limiting enzyme in hexosamine synthesis	Liver
L39373	Mannoside acetyl glucosaminyl transferase 3 (Mgat3); transfers the bisecting GlcNAc to the core of complex, N-linked carbohydrates	Liver
X72959	N-acetyl transferase 3 (Nat3)	Liver
J00355	Alpha-amylase-1; glycogen digestion and mobilization	Liver and salivary glands
J04947	Angiotensin converting enzyme (Ace); dipeptidyl carboxypeptidase that converts angiotensin I into the potent vasoconstrictor angiotensin II	Liver, brain
L09105	Glucose phosphate isomerase (GPI); a housekeeping gene expressed in all tissues and organisms that utilize glycolysis and gluconeogenesis.	Ubiquitous
ET62525	Polypeptide N-acetylgalactosaminyltransferase-T4 (polypeptide GalNAc transferase-T4; ppGaNTase-T4); fourth member of the mammalian UDP-GalNAc; Golgi-like localization; 4 GalNAc-transferase controls the initiation of mucin-type O-linked protein glycosylation	Ubiquitous
X14489	Thymidylate synthase (Tyms)	Ubiquitous (all proliferating cells)
U34071	Galactosidase, alpha (Gla); carbohydrate metabolism	Ubiquitous (most cases)

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
Cellular Component (cell adhesion/membrane components/extracelluar matrix)		
ET62381	K-cadherin/cadherin-6; present at external cell surface at cell-cell contact sites; calcium-dependent cell adhesion molecule	Brain (cerebral cortex in neonatal mice), thymocytes
X95226	Dystrobrevin (Dtn); formation and maintenance of mammalian neuromuscular junction	Brain (CNS)
X07215	Proteolipid protein (Plp), main integral protein of myelin	Brain (CNS)
ET61336	Nonmuscle myosin heavy chain IIB; cell motility	Brain (CNS)
ET63017	Cadherin 8 (Cdh8); adhesion molecule	Brain (subdivision of early CNS) and thymus
X57377	Myosin Va (Myo5a); cytoskeleton	CNS, cephalic ganglia, and spinal ganglia; lld skeletal myosin heavy chain gene expressed in fat-storing cells (FSC, lipocytes, or Ito cells) of regenerating liver
X66976	Procollagen, type VIII, alpha 1 (Col8a1); extracellular matrix: component of basal laminae	Epithelial, endothelial, and mesenchymal cells in newborn mouse tissue
M91602	Myosin light chain, phosphorylatable, cardiac ventricles (Mylpc); contractile protein, cytoskeleton	fat-storing cells (FSC, lipocytes, or Ito cells) of regenerating liver; muscle
ET63188	Fibroblast activation protein; cell-surface glycoprotein; member of the serine protease family; expressed at sites of tissue remodelling.	Fibroblasts
M17376	Alpha-1-acid glycoprotein I (AGP-1); membrane protein	Liver
U49185	Occludin (Ocln); occludin is a transmembrane protein located at tight junctions and is known to interact with other tight junction proteins	Liver
L02918	Procollagen type V alpha 2	Liver

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
V00830	Epidermal keratin subunit; intermediate filament protein; maintenance of epidermal cell shape and resistance to mechanical trauma	Liver (epithelial cells)
X53176	Integrin alpha 4 (Itga4); cell adhesion	Lymphocytes
X91043	Erythrocyte protein band 7.2 (Epb7.2); stomatin; involved in Na + /K + permeability of cells	Spleen, lung, testis, not reported in liver
U69136	Cadherin 9 (Cdh9); calcium-binding membrane glycoprotein; cell adhesion molecule	Thymocytes
X97227	CD53 antigen (Cd53); pan-leukocyte antigen; cell membrane glycoprotein	Thymocytes
X75636	Iduronato-2-sulfatase (Ids); degrades heparin sulfate and dermatan sulfate in lysosomes; deficiency causes fatal lysosomal storage disorder, mucopolysaccharidosis type II (the glycosaminoglycans heparin sulfate and dermatan sulfate accumulate); part of proteoglycans which bind, help package and store secretory molecules; function in cell adhesion and basal lamina formation	Ubiquitous
X66402	Matrix metalloproteinase 3 (Mmp3); extracellular matrix-degrading metalloproteinase	Ubiquitous
U56734	Mannose receptor, C type 2 (Mrc2); cell adhesion; antigen presentation	Wide tissue distribution
Cell surface receptor		
D78175	Natriuretic peptide receptor 3 (Npr3); membrane protein; modulates availability of natriuretic peptides at target organs; activation of G protein-coupled signaling system;	Epithelial and endothelial cells; lung (smooth muscle cells), heart (aortic smooth muscle cells)

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
M61000	Gastrin releasing peptide receptor (Grpr); member of the G protein-coupled receptor family	Fibroblasts
M35684	Complement receptor 2 (Cr2)	Late pre-B cells
ET61559	Bradykinin B1 subtype receptor; G protein-coupled membrane bound; T-kininogen modulation during acute phase protein synthesis	Liver (ubiquitous)
M86441	Fibroblast growth factor receptor 2 (Fgfr2); membrane-spanning tyrosine kinase; activated by three members of the FGF family	Liver parenchymal cells and others
U57612	CD44 antigen (Cd44); receptor for hyaluronan; cell surface glycoprotein; hyaluronan clearance from the blood; lymphocyte homing and activation	liver, CNS, other
ET61693	Leptin receptor (OB-R); transmembrane receptor	Liver, Lung, muscle, brain; developing bone, mesenchyme
ET62920	CC Chemokine Receptor-4; integral membrane protein; G-protein coupled receptor; signals involve chemotaxis and calcium flux; directs cell movement in thymus; directs monocytes and lymphocytes to their target tissues	Thymus, T cells, and monocytes
Molecular Motors:		
ET63395	Axonemal dynein heavy chain (mdhc1); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit motor ATPase complexes called dyneins	Brain, trachea, testis
ET63399	Axonemal dynein heavy chain (mdhc3); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit	Brain, trachea, testis

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	motor ATPase complexes called dyneins	
ET63402	Axonemal dynein heavy chain (mdhc6); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit motor ATPase complexes called dyneins	Brain, trachea, testis
ET63405	Axonemal dynein heavy chain (mdhc9); axonemal dyneins are molecular motors that drive the beating of cilia and flagella; heavy chains are main components of multisubunit motor ATPase complexes called dyneins	Brain, trachea, testis
ET62103	Nebulin; a family of giant myofibrillar proteins	
Serum Protein/Secreted Protein		
V00743	Alpha fetoprotein (Afp); main component of mammalian fetal serum; synthesized by visceral endoderm of the folk sac and by fetal liver; blood level decreases after birth; synthesis reactivated in liver tumors	Liver (fetal & adult)
Immune Cell Function/Primary Response Genes		
M88242	prostaglandin-endoperoxide synthase 2 (Ptgs2); putative mediator of inflammation; induced by growth factors and cytokines	Fibroblasts and monocytes
L38281	Immunoresponsive gene 1(Irg1); activated by bacterial LPS treatment	Macrophages
U43384	Cytochrome b-245, beta polypeptide (Cybb); a flavocytochrome that mediates the transfer of electrons from NADPH to molecular oxygen in the respiratory burst oxidase	Phagocyte

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
Y08026	Immunity-associated protein, 38 kDa (Imap38)	Spleen
X15592	Cytotoxic T lymphocyte-associated protein 2 beta (Ctla2b); homologue of cysteine protease proregion	T cells
Others		
ET62336	DNA ligase III-beta; DNA ligase III exists as two distinct isoforms denoted alpha and beta	Alpha is expressed in most tissues; beta is expressed in testes and during spermatogenesis
X61449	nucleosome assembly protein 1-like 1	Brain; expression poorly characterized
L28819	Involucrin (Ivl); a glycine-,serine- and cysteine-rich protein expressed late in differentiation of grandular layers in normal epidermis	Epidermis
X99251	Repetin (Rptn); calcium-binding; similar to intermediate filament-associated proteins profilaggrin and trichohyalin; expression during late epidermal differentiation	Epithelia of forestomach and tongue
ET61424	Protein-tyrosine phosphatase epsilon precursor; the protein tyrosine phosphatase epsilon (PTPepsilon) gene gives rise to two proteins: a transmembranal, receptor-like form and a cytoplasmic, non-receptor form	Hematopoietic tissues
U73915	Phosphate regulating neutral endopeptidases on the X chromosome (Phex); mineralization of extracellular matrix by osteoclasts	Kidney, bone
ET61364	Meprin beta subunit isoform (Mep-1 beta); meprins are membrane-bound oligomeric metalloendopeptidases, contain alpha and/or beta subunit	Kidney, intestine, not reported in liver
U60330	Proteaseome 3 (Psme3); Ki antigen; cell proliferation; enhances generation of class I binding peptides;	Liver, neurons, broad tissue distribution

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
X16490	Plasminogen activator inhibitor, type II (Planh2); serine protease inhibitor; inactivates urokinase-type plasminogen activator and regulates degradation of the extracellular matrix; one form is cytoplasmic the other is translocated into the endoplasmic reticulum	Liver; bone-marrow, spleen, lung, thymus, skin
X58169	T-complex protein 10a (Tcp10a); Tcp-10 gene has been established as a molecular candidate for the T complex responder locus which plays a central role in the transmission ratio distortion phenotype expressed by males heterozygous for a T haplotype.	Male germ line
Z46299	Sperm autoantigenic protein 17 (Sp17); sperm specific protein; calmodulin binding protein	Mammalian testis; sperm-specific
M26940	Casein beta (Csnb); milk protein	Mammary glands
ET63259	Cea14 gene (carcinoembryonic antigen family members); unknown function; member of the immunoglobulin superfamily	Many cea genes expressed in fetal liver
ET63260	Cea15 gen (carcinoembryonic antigen family members); unknown function; member of the immunoglobulin superfamily	Many cea genes expressed in fetal liver
ET63261	Cea16 gene (carcinoembryonic antigen family members); unknown function; member of the immunoglobulin superfamily	Many cea genes expressed in fetal liver
M20567	Heat shock protein, 70 kDa 2 (Hsp70-2); not induced by heat shock; developmentally regulated in P60spermatogenic cells; critical role in spermatogenesis	Meiotic phase of spermatogenesis
X04405	Myoglobin (Mb); small globular heme protein; oxygen-carrying	Muscle

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
ET63205	Odorant binding protein Ib	Nasal epithelium.
ET63156	Disabled homolog 1 (Drosophila) (Dab1); adaptor molecule in neural development	neuronal and hematopoietic cells
ET62968	Odorant receptor 23 (OR23)	Olfactory and testicular cells
U96701	Serine protease inhibitor 15 (Spi15); regulator of extracellular proteolysis	Predominantly in testis
ET63408	Capping protein beta 3 subunit; a novel isoform of actin-binding protein; a component of the cytoskeletal calyx of the mammalian sperm head.	Spermiogenesis
ET62832	Perforatorial protein (PERF 15); a novel testicular protein; sequence similarities to a family of lipid binding proteins; major component of the rat sperm perinuclear theca	Testis
Z38118	Synaptonemal complex protein 1 (Sycp1); pairing of chromosomes during meiosis	Testis
M19413	Tubulin alpha, related sequence 1 (Tuba-rs1)	Testis
Y08485	Synaptonemal complex protein 3 (Sycp3); part of the lateral element of the synaptonemal complex; a meiosis-specific protein structure essential for synapsis of homologous chromosomes	Testis; synaptonemal complex protein 1 is also expressed in embryonic ovary, adult brain and testis
X96737	Synaptobrevin like 1 (Sybl1); housekeeping gene; X-linked; inactivated on one X in every female cell, and also inactive on the Y of male cells	Ubiquitous
X92842	Surfeit gene 6 (Surf6); involved in a nucleolar ribosome maturation; housekeeping	ubiquitous (nucleolus)
ET62791	WW domain binding protein 6; WW domain is a globular protein domain that is involved in mediating protein-protein	Uncharacterized

208 known genes: 2-fold or greater in CR vs. Cont at old and young age

GenBank	Description	Location
	interaction and that ultimately participates in various intracellular signaling events; WW domain mediates protein-protein interaction by bin	
ET62978	Neosin/lark; RNA-binding protein; Drosophila homologue encodes an element of the clock output pathway regulating adult eclosion (circadian rhythm)	Uncharacterized, probably neuronal

APPENDIX H

142 known genes: 2-fold up in young CR vs young Control and unchanged in old CR vs old Control				
t-test	ng t-test	Bank	notype	ation
		Bank		
0.083	0.001	854	troglycan (Dag1); Dystrophin associated oprotein 1; acts as a receptor for ement membrane components	cle, epithelial cells (liver and others)
0.010	0.000	664	rin A5 (Efna5); Eph-related receptor sine kinase ligand 7; LERK-7; AL-1; S; essential for proper axon guidance and graphic mapping	ryo visual system, CNS and peripheral nervous system
0.084	0.014	122	assium inwardly-rectifying channel, family J, member 6 (Kcnj6) ; G protein-vated; play a role in resting potential and trollin excitabilit of the cell	rons
0.425	0.008	135 and 861	tin receptor (Lepr); Obr; leptin is a key ght control homone; mutation of the leptin ptor causes obesity	r, ubiquitous, but not in thymus or pancreas
0.346	0.008	701	inoblastoma-like 1(p107) (Rbl1); scriptional cell cycle repression; tumor pressor gene; binds to myc gene	hest in liver and heart embryo, lower in adult liver, CNS, iferating cells, heart, lung, kidney, intestine
0.117	0.000	5603	aete-scute complex homolog-like sophila) (Ascl1); helix-loop-helix protein scriptional factor; controls a basic ration in development of neuronal enitors in distinct neural lineages	eloping CNS and peripheral nervous systems, in adult n only
0.124	0.007	4120	ivin receptor IIB (Acvr2b); receptor for vins, which play an important part in oderm induction	ryo, testis
0.374	0.006	546	-ribosylation-like 4 (Arl4); ADP-sylation factor like protein 4; involved in ation of transport	pocytes, ubiquitous

			vesicles; expressed in erentiatina cells	
0.856	0.027	478	binding protein 1 (Aebp1); transcriptional essor with carboxylpeptidase activity; no scription during adipocyte differential or oblast calcification	eoblasts and adipose tissue
0.221	0.027	1705	aline phbsphatase 3, intestine, not Mn iring (Akp3); intestinal alkaline sphatase (IAP); a membrane-bound alloenzyme catalyzing cleavage of ganic phosphate nonspecifically from a e variety of phosphate esters.	stine, kidney
0.592	0.009	361 and 1891	ylase 2, pancreatic (Amy2); J00361 se alpha-amylase-like gene; glycogen stion and mobilization	creas, liver and many others
0.125	0.001	676	yloid beta (A4) precursor protein-binding, ily A, member 2 (Apba2); X11 protein e; X11 protein binds amyloid precursor ein; receptor trafficking; may regulate the essing of amyloid precursor protein to the loid beta peptide	rons
0.071	0.006	216	lipoprotein CII (Apoc2); required for lysis of triglycerides by lipoprotein lipase	I liver, adult liver, intestine and peritoneal macrophages
0.371	0.006	573	n derived neurotrophic factor (Bdnf); lates development and maintaince of the ous system	in, highest in hippocampus and cerebral cortex
0.062	0.004	740	ein delta (Csnd); epsilon-casein (milk ein)	mary glands
0.324	0.016	526	8 antigen (Cd48); BCM-1; Blast-1; iates cell adhesion	face of leukocytes
0.796	0.024	562	division cycle 25 homolog C (S. visiae) (Cdc25c); encodes nine/tyrosine phosphatases that activate lin-dependent kinases; control of sitions between phases of cell division	n spleen and thymus
0.101	0.039	715	ular retinoic acid binding protein 1 bp1); intracellular pipid binding protein a high affinity for retinoic acid	ely expressed during development also in thymus

0.051	0.006	690	omobox homolog 1 (Drosophila HP1 beta) x1); Homologous to Drosophila HP1 gene; its chromatin, rendering heritable changes gene expression; activates or silences es	quitous during development
0.541	0.002	032	lin B2 (Ccnb2); regulator of transitions een phases of cell division	hest in pachytene spermatocytes, also in early ryoenesis
0.343	0.002	771	ochrome c, testis (Cyct); maintains tinous spermatogenesis	tis
0.110	0.016	859	D (aspartate-glutamate-alanine-aspartate) polypeptide 4 (Ddx4); DNA helicase; ortant role in determination events of cells	ryonic gonads and testicular germ cells
0.059	0.034	3226	ensin related sequence cryptdin peptide eth cells) (Defcr-rs1); CRS1C; microbial peptide	eth cells of the small intestine; smooth muscle
0.044	0.031	903	a-like 1 homolog (Drosophila) (Dll1); cell-communication regulating the rmination of various cell fates during elopment	ryo, not in adult liver
0.167	0.038	986	mocollin 1 (Dsc1); a "skin-type" mosomal cadherin; formation of tinized epithelial structure during mouse elopement; cell to cell adhesion	ryo and skin
0.475	0.002	963	hanous homolog 1 (Drosophila) (Diap1); in regulation of cell morphology, adhesion cytokinesis; Rho regulates actin merization by targeting profilin via the hanous homolog 1 beneath specific ma membranes	oblasts (ubiquitous)
0.174	0.027	925	transcription factor 5 (E2f5); M.musculus mRNA for E2F-5 protein.	
0.899	0.000	328	-like module containing, mucin-like, hormone receptor-like sequence 1 (Emr1); M.musculus mRNA for 0.	
0.177	0.010	2930	hropoietin (Epo); M12930 Mouse erythropoietin gene, complete cds	
0.143	0.023	325	ogenital dysplasia homolog (Fgd1); U22325 Mus	

			musculus faciogenital dysplasis (Fgd 1) mRNA plete cds	
0.993	0.047	1737	specific gene 27 (Fsp27); M61737 M.musculus adiopocyte-specific mRNA, partial cds	
0.062	0.046	535	oblast growth factor 9 (Fgf9); U33535 Mus musculus fibroblast growth factor 9 (FGF-9) mRNA plete cds	
0.993	0.003	853	lin 1 (Fbln1); M.musculus (isolate Mk24) mRNA for the 3' end of MB-90/fibulin C form	
0.358	0.006	854	lin 1 (Fbln1); M.musculus (isolate Mk31) mRNA for MB-90/fibulin D form	
0.150	0.002	099	junction membrane channel protein beta 3 (Gjb3); connexin31	
0.341	0.000	953	olin (Gsn); J04953 Mouse gelsolin gene, complete cds	
0.956	0.001	265	osaminyl (N-acetyl) transferase 1, core 2 (Gcnt1); U19265 Mus musculus core 2-GlcNAc-transferase -GnT) mRNA, complete cds	
0.374	0.008	996	th factor receptor bound protein 10 (Gfb10); U18996 Mus musculus growth factor receptor-binding ein (Grb10) gene, complete cds	
0.145	0.041	0422	one 4 protein (Hist4); J00422 Mouse histoneH4 gene, complete cds	
0.724	0.000	071	eo box C5 (Hoxc5); U28071 Mus musculus homeobox_protein (Hoxc-5) gene, complete cds	
0.207	0.037	519	roxysteroid dehydrogenase-5, delta < 5 > -3-beta (Hsd3b5); Mus musculus 3-ketosteroid reductase D3b5) mRNA, complete cds	
0.116	0.003	973	rferon alpha family, gene 4 (Ifna4); X01973 Mouse gene for interferon alpha 4 (Mu IFN-alpha 4)	
0.095	0.007	599	rferon gamma receptor 2 (lfngr2); U69599 Mus	

			musculus interferon gamma receptor second chain r2) gene	
0.008	0.015	542	rleukin 6 (ll6);	
0.566	0.008	359	359 mouse alpha-amylase-2 gene	
0.086	0.009	761	oncogene (Jun); Mouse mRNA for protein homologous to human c-JUN	
0.785	0.046	08574	voltage-gated channel, subfamily S, 2 (Kcns2)Mus musculus potassium channel alpha subunit 9.2) mRNA, complete cds.	
0.128	0.018	193	tin complex 1, acidic, gene 10 (Krt1-10); L00193 Mouse epidermal keratin type I intermediate ent gene	
0.053	0.026	313	tin complex 1, acidic, gene 15 (Krt1-15); cytoskeletal structural protein D16313 Mouse cytokeratin gene, complete cds	
0.071	0.002	889	r cell lectin-like receptor, subfamily A, member 8 (Klra8); U12889 Mus musculus Ly49H mRNA plete cds	
0.763	0.002	4398	rin (Lor); M34398 Mouse loricrin mRNA, complete cds	
0.088	0.016	503	phoid enhancer binding factor 1 (Lef1); D16503 Mouse mRNA for LEF-1S, complete cds	
0.079	0.001	3099	usculus epidymal sperm gene	
0.701	0.026	3121	usculus mRNA for alpha tectorin	
0.016	0.019	3404	usculus mRNA for axonemal dynein heavy chain (partial, ID mdhc8)	
0.145	0.003	3397	usculus mRNA for cytoplasmic dynein heavy chain (partial, ID mdhc11).	
0.591	0.000	3151	usculus mRNA for neural cell adhesion ecule	
0.119	0.047	3209	usculus mRNA for Nkx2-3 gene	
0.225	0.040	3083	usculus PR264 gene	
0.208	0.034	595	gen activated protein kinase kinase kinase kinase	

			2 (Map4k2); U50595 Mus musculus Rab8- ractin protein mRNA, complete cds	
0.139	0.000	3431	se DNA for neurotrophic factor, exon 3 and complete cds	
0.044	0.039	3429	se DNA for vav-T, partial cds	
0.392	0.032	997	se mRNA for cytotoxic T-cell membrane glycoprotein Ly-3 3'flank	
0.153	0.028	1015	se NLRR-2 mRNA for leucine-rich-repeat protein, partial cds	
0.427	0.015	210	musculus (Notch2) mRNA, complete	
0.000	0.034	2373	musculusACF7 neural isoform1 (mACF7) mRNA, partial cds	
0.066	0.000	3257	musculus cea12 gene	
0.087	0.028	3255	musculus cea9 gene	
0.302	0.034	1200	musculus complement recepter (CRY) mRNA, partial cds (spleen- specific)	
0.170	0.000	1528	musculus CRE-BP1 transcription factor, novel sliced form, mRNA, partial cds	
0.189	0.043	2694	musculus glucose-6-phosphate dehydrogenase (G6PD) gene, nuclear gene encoding mitochondrial ein, exon 6 and partial cds	
0.374	0.000	1692	musculus implantin mRNA, partial cds	
0.142	0.018	2692	musculus laminin alpha 3B chain (Lama3B) mRNA, partial cds	
0.346	0.005	2477	musculus mena protein (Mena) mRNA, complete cds	
0.155	0.000	1218	musculus microtubule-associated protein 4 (MAP4) mRNA, partial cds	
0.075	0.001	1544	musculus P- glycoprotein (mdr2) gene, , partial cds	
0.084	0.040	2235	musculus polyreactive autoantibody, immunoglobulin IgM heavy chain mRNA,	

			partial cds	
0.074	0.001	1683	musculus potassium channel mKv3.2 mRNA, partial cds	
0.043	0.019	1621	musculus putative protein kinase MRK2 mRNA, partial cds	
0.110	0.026	1556	musculus rearranged T cell receptor (TCRV-alpha-22.1) mRNA, variable region, partial cds	
0.191	0.026	2586	musculus sodium channel 2 (mBNaC2) mRNA, partial cds	
0.104	0.033	2280	musculusTcell receptor V alpha mRNA, partial cds	
0.459	0.003	1439	musculus trp-related protein 2 mRNA, partial cds	
0.061	0.002	1441	musculus trp-related protein 5 mRNA, partial cds	
0.054	0.004	0474	istoylated alanine rich protein kinase C substrate (Macs); M60474 Mouse myristoylated alanine-rich inase substrate RCKS) mRNA, complete cds	
0.378	0.017	360	plastic progression 1 (Npn1); M.musculus (Balb/C) P/L01 mRNA	
0.153	0.000	919	roblastoma myc-related oncogene 1 (Nmyc1); Mouse N-myc gene	
0.827	0.049	819	ropeptide Y receptor Y1 (Npylr); D63819 Mouse mRNA for neuropeptide Y-Y1 receptor, complete	
0.186	0.000	0514	al (Nodal); X70514 M. musculus nodal gene, a TGF-beta-like gene	
0.133	0.046	163	in (Nog);	
0.238	0.029	033	lear protein 220 (Np220); Mouse mRNA for nuclear protein, NP220, complete cds	
0.062	0.000	804	rnall expressed gene 3 (Peg3); U48804 Mus musculus Zn-finger protein Pw1 gene, complete cds	
0.348	0.030	687	nylethanolamine-N-methyltransferase (Pnmt);	

			L12687 Mouse phenylethanolamine N-hyltransferase gene, complete cds	
0.307	0.005	279	sphatidylinositol 3-kinase, catalytic, alpha polypeptide (Pik3ca); U03279 Mus musculus Balb/c sphatidylinositol 3-kinase 110 kDa subunit_mRNA, complete cds	
0.233	0.046	277	spholipase A2 group VII (platelet-activating factor acetylhydrolase, plasma) (Pla2g7); U34277 Mus culus PAF acetylhydrolase mRNA, complete cds	
0.408	0.000	305	ssium voltage gated channel, shaker related subfamily, member 1 (Kcna1); Mouse MBK1 mRNA for se brain potassium channel protein-1.	
0.958	0.038	789	iferation-associated protein 1 (Plfa); M.musculus mRNA for p38-2G4.	
0.063	0.000	594	iferin related protein (Plfr); Mouse mRNA for proliferin-related protein (PRP).	
0.146	0.009	532	ein kinase C, beta (Pkcb); Mouse mRNA for protein kinase C beta-II.	
0.109	0.009	242	ein kinase C, eta (Pkch); D90242 Mouse mRNA for nPKC-eta	
0.188	0.047	577	ein kinase C, lamda (Pkcl); Mouse mRNA for protein kinase C lambda	
0.196	0.049	935	ein kinase, cAMP dependent regulatory, type II alpha (Prkar2a); J02935 Mouse cAMP-dependent ein kinase type II regulatory subunit mRNA, 3' end	
0.542	0.011	720	ein that interacts with C kinase 1 (Pick1); M.musculus mRNA for perinuclear binding protein	
0.374	0.013	768	eoglycan 2, bone marrow (Prg2); L46768 Mus musculus major basic protein (MBP-1) gene,	

			complete	
0.256	0.013	133	eoglycan, secretory granule (Prg); Mouse mRNA for mastocytoma proteoglycan core protein, lycin	
0.904	0.022	239	5B, member RAS oncogene family (Rab5b); X84239 M.musculus mRNA for rab5b protein	
0.010	0.027	247	-related C3 botulinum substrate 2 (Rac2); M.musculus EN-7 mRNA	
0.115	0.034	711	ication factor C, 140 kDa (Recc1); M.musculus mRNA for replication factor C, large subunit	
0.463	0.023	642	finer protein (C3HC4 type) 19 (Rnf19)X71642 M.musculus GEG-154 mRNA	
0.694	0.021	5732	inal vesicle protein 2 (Svp2); Mouse seminal vesicle secretory protein IV (SVS IV) mRNA, 3' end	
0.714	0.032	7790	m amyloid A pseudogene (Saa-ps); M17790 Mouse SAA4 gene encoding serum amyloid A, exons 34	
0.009	0.021	580	en in absentia 1B (Siah1b); M.musculus siah-1B protein mRNA	
0.103	0.021	687	ium channel, voltage-gated, type I, beta polypeptide: (Scn1b); L48687 Mus musculus voltage-endent Na+ channel beta-1 subunit gene, exons 4-6	
0.328	0.002	268	te carrier family 35 (CMP-sialic acid transporter), member 1 (Slc35a1); M.musculus mRNA for CMP-ic acid transporter	
0.114	0.037	287	ulated by retinoic acid gene 8 (Stra8); M.musculus mRNA for Stra8 protein	
0.043	0.007	749	interactin factor (Tgif); X89749 M.musculus mRNA for mTGIF protein	
0.394	0.002	6987	scription factor CP2 (Tcfcp 2); Mouse alpha-globin transcription factor CP2 mRNA sequence	

0.177	0.034	362		
0.402	0.001	462	sforming growth factor, beta 1 (Tgfb1); L42462 Mus musculus TGF-1 gene, promoter region and	
0.533	0.004	534	or necrosis factor receptor superfamily, member 18 frsf18); Mus musculus glucocorticoid induced TNFR ily related protein precursor, mRNA, complete cds	
0.306	0.013	581	uitin-activating enzyme E1, Chr X (Ube1x); ubiquitin-dependent protein degradation	
0.438	0.006	859		
0.172	0.005	361	oncogene (Vav); X64361 M.musculus vav mRNA	
0.110	0.048	01598	icular inhibitor amino acid transporter (Viaat);	
0.008	0.019	438	entin (Vim);	
0.124	0.041	434		
0.495	0.001	9797	gless-related MMTV integration site 4 (Wnt4); M89797 Mouse Wnt-4 mRNA, complete cds	
0.056	0.031	496	496 Murine H3.1 gene for histone H3.1	
0.567	0.010	174	finger protein 30 (Zfp30);-Z30174-M.domesticus (C57B1/6J) mRNA for zinc finger protein 30	

APPENDIX I

known genes: 2-fold up in old CR vs. old Control and unchanged in young CR vs. young Control

	R 1	R 2	R 3	ont 7	ont 8	ont 9	*st*	ngCR 16	ngCR 17	ngCR 18	ngCont 19	ngCont 20	ngCont 21	*st*	notype	*ue distribution*
602	8.75	11.48	3.04	0.00	0.00	0.00	***0.003***	0.00	0.00	29.61	0.00	0.00	0.00	***.374***	e morphogenetic tein 2 (Bmp2); is component of a e-derived extract ical to initiation of ation of cartilage its conversion bone; is cturally related ransforming wth facto-beta s, vins, and bins. BMP aling is essential development of letogenic and rogenic cranial ral crest.	oblastic cells; or types of -hematopoietics (these cells uded oblasts, tinocytes, ocytes, kidney helial cells, and or cells of e, muscle, , liver, kidney, ach, colon, state, and ronal tissue)
3463	2.96	1.91	0.92	0.00	0.00	0.00	***0.031***	2.38	1.43	1.49	1.08	0.62	0.00	***.052***	YDROXYACYLSPHINGOSINE 1-A-GALACTOSYLTRANSFERASE CURSOR (EC 2.4.1.45) (UDP-LACTOSE-CERAMIDE LACTOSYLTRANSFERASE) RAMIDE UDP-LACTOSYLTRANSFERASE) REBROSIDE SYNTH	
2773	1.04	0.96	1.92	0.16	0.00	0.00	***0.016***	1.99	2.96	1.05	0.00	0.00	1.77	***.155***	HRIN-A3 (EPH-RELATED EPTOR TYROSINE KINASE AND 3) (LERK-3) (EHK 1 LIGAND) K1-L) (FRAGMENT).	
1172	2.83	10.69	9.99	0.00	0.00	0.00	***0.000***	8.70	0.00	2.72	1.52	0.00	0.00	***.347***	CYTOKINE RECEPTOR CURSOR (EC 2.7.1.112) ROSINE-PROTEIN KINASE EPTOR FLK-2) (FETAL LIVER ASE 2) (TYROSINE-PROTEIN	

															ASE FLT3).	
2049	2.43	10.05	8.49	0.00	0.00	0.00	***0.040***	3.04	55.00	0.00	0.00	0.00	0.00	***.243***	MEOBOX TEIN HOX-D3	X-4.1) (MH-19).
971	3.57	30.24	5.17	0.00	0.00	0.00	***0.031***	1.19	0.00	2.87	0.00	0.00	18.88	***.598***	ptor protein plex AP-2, alpha bunit (Ap2a1); gi-plasma brane transport icle; intracellular tein traffic; iates protein ing in the ocytic and late retory pathways	uitou
940	9.37	62.71	0.48	0.00	0.00	0.00	***0.020***	4.54	47.82	0.00	0.00	0.00	12.28	***.445***	aragine thetase (Asps); scription is ced by amino and ohydrate rivation	r
0903	2.38	2.80	1.39	0.00	0.52	0.00	***0.011***	0.00	0.51	0.61	1.61	2.13	2.56	***.007***	mphoid kinase); a member of family of tooncogenes; brane- ociated protein sine kinase; ction in B- phocyte specific sduction hway	ressed cifically in cells he B-lineage, in e pro-B cells in most pre-B mature B cells, not in plasma s
019	1.31	0.96	1.29	0.00	0.56	0.58	***0.022***	1.04	0.87	1.44	1.13	0.72	1.25	***.726***	igin (Bsg); 019 Mouse gene basigin precursor, igin signal ursor	
991	0.78	1.33	1.43	0.34	0.58	0.37	***0.025***	1.12	1.84	1.91	0.00	0.88	1.83	***.289***	tonin (Calc)	roid gland
343	5.03	16.01	1.89	0.00	0.00	0.00	***0.001***	0.00	0.00	27.69	0.00	0.00	0.00	***.374***	ium channel beta	uitou

															bunit (Cacnb2); tage-sensitive ium channels are ely expressed plexes which e both trogenic and al transduction ctions.	
966	2.24	2.14	1 .72	0.83	1.05	0.52	***0.005***	0.80	1.47	0.95	0.88	0.80	2.05	***.725***	onyl reductase 1 r1);a cytosolic ber of the aldo- reductase group nzymes. uitous enzyme abolize a variety ompounds taining carbonyl ups.	uitou
079	0.83	1.18	1.02	0.54	0.51	0.33	***0.010***	1.38	0.98	1.19	0.69	1.71	1.17	***.994***	dermal-neural ex 1 9 (Enc1); an y and highly cific marker of ral induction in ebrates; encodes Ich family related tein that is ; ENC- nctions as an n-binding protein t may be ortant in the anization of the n cytoskeleton ing neural fate cification and elopment of the	ryogenesis
320	8.37	14.30	8.47	0.00	0.00	0.00	***0.046***	0.00	0.00	0.00	7.15	0.00	0.00	***.374***	structure cific onuclease 1	

															1); a structure-cific onuclease,	
409	1.15	1.66	1.46	0.12	0.84	0.20	***0.019***	1.70	1.72	0.85	0.00	1.34	0.22	***.148***	head box A2 a2); transcription or	r, endoderrn-ved tissues, stomach, an ll intestine.
023	1.55	1.31	1.38	0.79	0.31	0.61	***0.006***	0.37	1.69	0.96	0.00	1.08	1.04	***.595***	eral transcription or IIH,	
															peptide 1 (62kD unit) (Gtf2h1);	
703	3.13	33.12	5.39	0.00	0.00	0.00	***0.031***	2.28	21.40	7.63	0.00	0.00	0.00	***.141***	-Kruppel family member GL12 2); Z22703 M.musculus	
															tinocyte growth factor Fgf-7	
255	1.25	1.99	2.59	0.00	0.59	0.00	***0.016***	1.44	0.75	1 .26	0.00	1.27	0.00	***.197***	-Kruppel family ber GLI3 musculus mRNA	
															Gli3 protein.	
498	1.47	1.76	1.46	0.70	0.87	0.66	***0.002***	0.74	1.04	0.96	0.91	1.30	1.53	***.178***	amate cysteine se (gamma-amylcysteine thetase), catalytic Ic);	r, embryo
498	5.92	22.45	4.96	0.00	0.00	0.00	***0.039***	5.05	1.71	28.66	0.00	0.00	0.00	***.236***	amate receptor, tropic, AMPA2 ha 2) (Gria2);	
754	9.29	15.43	1.98	0.00	0.00	0.00	***0.002***	7.98	18.64	0.00	0.00	0.00	0.00	***.116***	t shock factor 2 f2)M.musculus	
															NA for heat shock scripton factor 2.	
0365	2.12	2.48	2.79	0.00	1.29	0.00	***0.013***	2.69	0.91	0.37	1.09	0.00	0.00	***.291***	tion plakoglobin (Jup); one of the teins of desmosomal membrane horage site plaques of the helium, and is also acomponent of	
239	5.76	13.54	7.04	0.00	0.00	0.00	***0.022***	2.55	0.00	26.67	0.00	0.00	0.00	***.316***	ues of the adherins junction uscsulus mRNA	

														goosecoid eobox.
778	1.17	17.10	8.21	0.00	0.00	13.02	***0.032***	9.71	2.14	0.00	0.00	0.00	0.00	***.251*** usculus H2B e.
889	0.94	1.46	1.95	0.65	0.00	0.55	***0.041***	1.42	1.73	0.91	0.70	2.19	1.06	***.950*** usculus mRNA wnt-8D protein.
796	6.71	6.58	0.91	0.00	0.00	0.00	***0.046***	3.72	13.92	38.64	0.00	.0.00	0.00	***.056*** usculus mRNA ologous to S. visiae RAD54.
942	4.93	4.23	1.66	0.55	0.00	1.26	***0.047***	4.85	0.74	4.66	0.00.	0.68	0.44	***.088*** usculus mRNA nhancer-trap- s 1.
060	3.39	1.14	1.65	0.18	0.00	0.00	***0.043***	0.86	2.53	2.91	8.19	0.00	0.00	***.834*** usculus myf-6 e.
2974	2.34	1.55	1.44	0.00	0.00	0.00	***0.003***	1.35	1.03	2.14	0.97	0.69	0.00	***.096*** usculus SOX1 e.)PIR: S 10950 -determining tein - mouse gment)
3453	6.48	30.32	7.19	0.00	0.00	0.00	***0.009***	9.94	4.23	2.36	0.00	0.00	0.00	***.273*** 3453 Mouse somal protein ' (rpL32') gene, plete cds
885	1.34	1.23	2.44	0.20	0.84	0.00	***0.046***	0.00	0.00	5.53	2.69	1.16	0.00	***.794*** tocytoma N-deacetylase/N- otransferase (Mndns); usculus mRNA for glucosaminyl eacetylase / N-sulfotransferase.
461	2.63	1.67	1.27	0.40	0.00	0.00	***0.015***	1.26	1.84	0.81	0.00	1.19	0.00	***144*** rine Hox2.2 NA for a eobox protein.
1617	1.41	1.14	1.53	0.86	0.25	0.57	***0.019***	1.18	1.72	1.38	0.76	0.71	0.73	***.012*** s musculus 5E6 6/Ly-49C) mRNA, plete cds.
2456	1.22	9.34	6.07	0.00	0.00	0.00	***0.036***	3.70	0.00	0.00	4.26	0.00	0.00	***.420*** s musculus in-2 mRNA, ial cds.
2752	1.25	1.27	0.96	0.71	0.28	0.75	***0.032***	1.26	1.14	1.18	0.81	0.55	1.04	***.053*** s musculus tocyst unknown tein mRNA, partial

3262	0.84	1.16	1.18	0.65	0.00	0.19	***0.025***	1.32	1.29	1.36	1.27	0.04	0.73	***.143***	s musculus cea17 e.
2465	1.51	1.15	0.99	0.48	0.00	0.66	***0.029***	1.82	1.34	1.01	0.68	0.79	2.48	***.913***	s musculus erbB2 NA, partial cds.
737	1.13	0.87	1.16	0.00	0.76	0.00	***0.041***	1.26	1.33	0.00	1.41	1.27	0.80	***.557***	s musculus ne/threonine- tein kinase 4m (PRP4m) NA, complete cds
889	1.50	1.60	1.40	0.55	0.83	0.51	***0.002***	1.02	1.14	1.01	0.99	0.47	0.88	***.168***	s musculus SH3- taining protein P3 mRNA, partial
620	1.83	9.81	3.90	0.00	0.00	0.09	***0.023***	1.48	0.52	0.00	8.09	0.00	14.09	***.177***	s musculus I/SNF complex 60 subunit (BAF60
2078	0.90	1.03	1.23	0.37	0.18	0.80	***0.043***	1.21	1.47	1.09	0.62	1.64	0.97	***.607***	s musculus scription factor 4 (tbx4) mRNA, ial cds.
283	2.27	0.80	1.93	0.11	0.45	0.52	***0.048***	1.15	0.85	0.31	1.49	1.99	3.51	***.076***	s musculus scription factor 2 (USF2) gene
247	1.20	13.60	8.56	0.00	0.00	0.00	***0.001***	2.78	19.06	0.00	0.00	0.00	30.03	***.826***	s mUSCUIUS zinc er protein (kid-1) e, complete cds.
128	3.25	3.67	2.03	0.00	0.00	1.82	***0.038***	0.00	0.00	0.00	0.18	3.26	6.55	***.144***	***assium*** voltage-gated channel, family H (eag-related), member 2 nh2); Mus musculus ether-a-go- elated protein isoforrn Mergi a rg1) mRNA, complete cds.
3227	1.35	1.40	1.47	0.60	1.05	0.22	***0.031***	0.70	0.95	0.63	0.61	1.78	1.91	***.189***	roenkephalin 2 ells k2);a homolog of brain
2136	2.63	30.79	0.41	0.00	0.00	0.00	***0.001***	0.00	51.41	43.25	0.00	0.00	0.00	***.119***	roenkephalin, a rotransmitter collagen type IX, alpha o19a1); a fibrillar collagen, the ely distributed elements of the acellular matrix.

405	1.12	1.22	1.32	0.00	0.88	0.06	***0.036***	1.52	0.30	2.07	0.00.	0.00	2.18	***.558***	collagen, type VI, a 1 (Col6a1);
320	1.15	1.31	1.26	0.00	0.44	0.61	***0.009***	1.20	1.30	1.06	0.00	0.39	0.94	***.057***	kinje cell protein 4 n 4);
429	4.85	13.23	5.19	0.00	0.00	0.00	***0.047***	0.64	0.00	0.00	0.00	0.00	0.00	***.374***	21 homolog (S. pombe) d21); 049429 Mouse P-29 mRNA for PW29, plete cds
071	1.10	1.20	1.12	0.73	0.26	0.70	***0.021***	0.92	0.83	1.45	0.80	1.08	1.10	***.750***	-related orphan ptor gamma rc); r, muscle, ey, brain, lung, is, and spleen.
506	2.15	1.49	1.34	0.96	0.78	0.12	***0.044***	0.34	1.51	0.07	0.20	1.61	1.04	***.634***	binding motif tein 4 (Rbm4);
921	2.76	2.75	2.49	0.31	0.90	0.00	***0.001***	1.10	0.00	2.28	0.00	1.38	0.05	***.461***	0 calcium-binding protein A 13 00a13); M.musculus mRNA for 0 calcium-binding protein A13.
262	3.53	2.20	2.52	0.33	0.22	0.17	***0.003***	1.45	0.55	2.37	0.26	1.70	0.00	***.341***	solute carrier family 1, member (Slc1a6); a Purkinje-cell specific neuronal glutamate transporter
542	1.58	1.23	1.42	0.85	0.00	0.00	***0.020***	0.57	1.61	1.68	0.00	0.71	1.15	***.247***	ocerebellar ataxia 1 homolog an) (Sca1) M.musculus mRNA ataxin-1.
184	1.22	8.76	8.97	0.00	0.00	0.00	***0.013***	3.50	0.00	2.35	2.67	0.00	0.00	***.360***	ell expressing clone j6 (Tj6); usculus J6B7 mRNA for T cell duced protein.
796	1.68	8.76	8.91	0.00	0.00	0.00	***0.000***	2.27	24.48	0.00	0.00	0.00	0.00	***.117***	is specific X- ed gene (Tsx);
861	2.82	1.65	2.44	0.82	0.81	0.88	***0.013***	1.02	1.00	0.65	0.63	1.00	1.46	***.626***	1 cytotoxic ule-associated -binding protein- 1 (Tial1); ptosis
4429	1.32	1.12	1.18	0.43	0.80	0.00	***0.029***	1.20	1.03	0.97	0.40	0.46	2.07	***.872***	scriptional rmediary factor lpha (Tif1a);
167	0.77	1 .50	1.38	0.20	0.14	0.64	***0.032***	1.23	1.26	1.51	1.60.	0.01	0.00	***.213***	sient receptor tein 1 (Trrp 1);
697	2.55	3.62	3.28	0.00	0.00	0.00	***0.001***	2.71	3.07	0.00	0.00	0.00	2.33	***.407***	oil factor 2 (spasmolytic protein Tff2); M.musculus spasmolytic

															peptide (SP) mRNA.
960	1.87	1.22	0.84	0.00	0.00	0.14	***0.015***	1.03	0.00	1.18	3.37	3.78	0.97	***.107***	sine kinase ptor 1 (Tie1); usculus mRNA TIE receptor sine kinase.
297	0.99	1.46	1.35	0.00	0'.00	0.00	***0.001***	1.26	1.32	1.01	0.59	0.14	1.06	***.100***	297 Mus culus chrome 8561 yt) mRNA, plete cds
399	6.64	22.65	5.57	0.00	0.00	0.00	***0.001***	4.45	42.28	9.23	0.00	0.00	0.00	***.099***	399 Mus culus Cdk- bitor p57KIP2 2) mRNA, plete cds
788	1.60	1.19	2.04	0.00	0.00	0.00	***0.003***	1.25	0.92	1.93	0.35	0.00	1.08	***.111***	788 Human ative cytochrome c- e synthetase NA, complete cds
818	1.30	1.21	0.93	0.79	0.09	0.52	***0.043***	1.07	0.68	0.51	1.45	2.13	1.88	***.014***	818 Mus culus tuberin C2) mRNA, plete cds
925	1.35	1.33	0.87	0.43	0.50	0.50	***0.012***	0.13	1.13	1.49	1.27	1.83	0.00	***.871***	925 Mus culus scription factor mRNA, complete
085	1.43	1.51	0.76	0.00	0.00	0.47	***0.020***	1.40	1.24	1.56	0.00	0.00	8.39	***.645***	085 Mus culus thiazide- sitive Na-Cl ransporter mRNA, plete cds
673	2.43	25.66	2.46	2.34	0.00	0.00	***0.028***	6.97	0.00	0.00	0.00	0.00	215.85	***.460***	673 Mus musculus histone (A)-613, histone H2a(B)-613, and one H2b-613 (H2b) genes, plete cds
849	2.21	2.41	1.17	1.06	0.00	0.00	***0.039***	1.76	0.58	0.94	0.80	0.29	2.39	***.928***	849 Mouse

998	1.75	1.67	1.29	1.03	0.65	0.36	***0.022***	0.83	1.38	1.51	0.24	0.00	0.97	***.080***	kFGF genomic 998 M.musculus for gamma 2c
438	1.25	1.54	1.20	0.00	0.00	0.00	***0.000***	0.80	3.02	1.36	0.15	0.00	1.56	***.236***	438 Mouse MP4 e for a proline- protein
398	8.81	54.55	5.19	0.00	0.00	0.00	***0.007***	9.14	12.37	4.19	0.00	0.00	0.00	***.051***	398 Mouse Flt3 NA for tyrosine se receptor of PDGF
091	1.09	1.23	1.06	0.54	0.00	0.67	***0.027***	1.00	1.19	1.05	0.41	0.00	1.00	***.106***	091 M.musculus mRNA
719	1.51	1.33	1.41	0.00	0.00	0.13	***0.000***	1.25	0.84	1.19	1.16	0.00	0.27	***. 177***	719 Mus culus CREB gene cAMP-responsive- ent binding tein, exon 2
904	1.21	2.04	1.39	0.00	0.96	0.00	***0.039***	1.97	3.00	1.04	0.00	0.07	0.82	***.052***	904 M.musculus A5T mRNA for T receptor alpha in
850	2.50	1.83	1.79	0.00	0.00	0.00	***0.001***	0.00	8.14	0.52	0.00	1.48	6.28	***.939***	850 M. culus mRNA for P kinase- vated protein se 2
104	7.34	15.22	7.66	0.00	0.00	0.00	***0.029***	6.59	11.41	0.00	0.00	0.00	0.00	***.175***	104 M.musculus NA for gli2 gene
677	3.74	25.02	4.42	0.00	0.00	0.00	***0.008***	0.00	79.97	0.00	0.00	0.00	0.00	***.374***	aguchi sarcoma I (v-yes) ogene hoinolog s);
509	1.18	2.10	1.25	0.46	0.00	0.72	***0.037***	1.18	1.23	2.33	0.72	0.00	0.82	***.079***	509 Mouse NA for PAP
544	0.96	1.28	1.67	0.83	0.21	0.34	***0.039***	0.54	1.04	1.14	1.25	0.19	1.20	***.952***	ologous protein use mRNA for ase small unit, complete
801	1.99	1.90	1.93	0.00	0.00	0.00	***0.000***	1.41	2.04	0.59	0.00	0.00	2.64	***.657***	801 Mouse

095	1.34	1.23	1.43	0.52	0.14	0.15	***0.002***	1.00	2.10	1.00	0.85	1.25	0.90	***.398***	NA for Emb, plete cds 095 Mouse DNA
471	5.32	27.16	8.38	0.00	0.00	0.00	***0.027***	2.16	27.48	17.72	0.00	0.00	0.00	***.099***	histamine H 1 ptor, complete 471 House se; Musculus esticus testis
900	1.35	1.66	1 .86	0.06	0.00	1.18	***0.043***	0.63	1.81	0.82	0.00	1.39	0.00	***.351***	NA for gsg3, plete cds 900 House se; Musculus esticus male
146	6.21	18.86	8.67	0.00	0.16	0.00	***0.000***	6.27	25.66	1.84	0.00	0.00	0.08	***.199***	n mRNA for 3, complete cds 146 Mouse
1090	5.84	5.50	7.16	0.00	0.00	0.00	***0.000***	2.92	14.39	7.89	0.00	0.00	0.00	***.065***	9d gene use DNA for
1642	1.28	1.60	1.43	0.25	0.00	0.00	***0.000***	1.73	1.46	0.94	1.06	0.00	0.31	***.078***	odine receptor e-3, exon 2, ial cds. ROTEIN-ACTIVATED INWARD
1677	3.66	3.39	4.44	0.00	0.00'	0.00	***0.000***	2.77	2.79	0.93	0.00	0.00	1.07	***.065***	TIFIER POTASSIUM CHANNEL 2 K2) (POTASSIUM CHANNEL, ARDLY RECTIFYING, SUBFAMILY EMBER 6) (KIR3.2). ILORIDE-SENSITIVE SODIUM
2211	1.51	1.55	1.09	0.84	0.47	0.24	***0.019***	1.42	0.91	1.32	0.29	0.45	1.09	***.904***	NNEL ALPHA-SUBUNIT (LUNG CHANNEL ALPHA SUBUNIT) PHA ENAC) (NONVOLTAGE-TED SODIUM CHANNEL 1 ALPHA UNIT) (SCNEA) (ALPHA NACH) AGMENT). s musculus
2229	1.20	1.67	1.04	0.00	0.00	0.00	***0.002***	1.23	1.68	1.36	0.55	0.96	0.94	***.031***	ant formin (Fmn) e, partial cds. s musculus t10b mRNA,

														plete cds.
2444	2.44	1.02	0.98	0.00	0.00	0.00	***0.037***	1.41	1.71	2.08	0.00	1.14	0.94	***.060***	s musculus Sox4 x4) mRNA, partial
2446	9.81	28.01	8.98	0.00	0.00	0.00	***0.000***	9.16	15.36	0.29	0.00	0.00	0.00	***.182***	s musculus Sox 12 x12) mRNA, ial cds.
2570	1.20	1.21	1.81	0.60	0.00	0.40	***0.016***	0.80	2.67	3.46	0.00	0.23	1.22.	***105***	s musculus Mad olog Smad5 NA, complete
2673	1.24	1 .58	1.28	0.35	0.93	0.00	***0.033***	1.74	0.41	0.70	1.76	1.07	0.26	***.900***	use hyaluronan thase 3 mRNA, plete cds.
2998	0.93	1.07	1.36	0.07	0.00	0.00	***0.001***	1.19	1.08	1.34	0.51	0.01	1.21	***.153***	usculus mRNA dystrobrevin ne m32).
3005	1.98	1.19	0.81	0.00	0.00	0.00	***0.018***	0.00	2.97	1.35	3.07	5.28	0.00	***.487***	usculus mRNA phospholipase C ma 1.
3019	0.61	1.69	1.47	0.00	0.39	0.00	***0.033***	1.39	1.55	1.93	0.00	1.43	0.00	***.086***	usculus skeletal cle ryanodine ptor gene.
3122	1.10	1.77	1.18	0.62	0.51	0.52	***0.021***	0.84	1.19	1.52	1.33	0.74	0.90	***.515***	usculus mRNA beta tectorin.
3226	1.13	1.26	1.06	0.59	0.30	0.64	***0.006***	1.02	0.98	1.05	0.47	0.79	1.15	***.331***	usculus htlf e, exon.
3241	1.07	1.17	0.98	0.51	0.45	0.39	***0.001***	1.69	1.02	1.23	0.52	0.63	1.60	***.370***	OPAIN PRECURSOR (EC 3.4.22.-) STEINE PROTEASE CPP32) MA PROTEIN) (CASPASE-3) E).
3410	1.13	1.60	1.69	0.36	0.58	0.00	***0.009***	1.05	1.19	1.25	0.04	0.95	0.46	***.065***	usculus mRNA semaphorin Hv 88 bp).
3528	1.25	1.34	1.14	0.43	0.17	0.18	***0.001***	1.00	1.11	1.00	0.29	0.00	1.19	***.207***	s musculus A-protein (A-myb) e, partial cds.
293	8.72	10.87	2.41	0.00	0.00	0.00	***0.001***	0.00	2.18	0.00	8.26	2.35	0.00	***.335***	use skeletal cle sphorylase se, gamma

														unit mRNA, plete cds
298	1.25	1.49	1.34	0.00	0.76	0.89	***0.046***	1.64	1.08	1.06	0.94	0.80	0.36	***.095***	298 Mouse rine otransferrin NA
060	6.51	7.40	5.77	2.15	0.00	0.00	***0.002***	3.72	7.47	0.00	0.00	0.00	0.00	***.159***	060 Mouse somal protein, e 3A coding for
785	1.75	0.96	1.61	0.73	0.26	0.00	***0.027***	2.30	1.04	1.75	0.65	1.40	0.00	***.138***	use mRNA ced by PDGF h some homology -fos.
928	2.01	3.91	3.04	0.00	1.45	0.00	***0.027***	5.18	0.00	0.00	1.79	0.55	0.23	***.654***	s musculus erentiation igen (CD22) NA, complete
472	1.38	1.81	1.41	0.00	0.52	0.00	***0.004***	1.84	0.70	1.23	1.50	0.35	0.77	***.458***	472 Mouse Bax a mRNA, plete cds
167	1.05	1.39	1.67	0.51	0.00	0.00	***0.008***	1.89	0.95	1.43	0.00	1.86	0.00	***.299***	167 Mouse zinc er protein mRNA
580	2.58	18.21	9.09	0.00	0.00	0.00	***0.004***	7.22	42.38	12.47	0.00	0.00	0.00	***.132***	580 Mus culus galanin e
567	2.83	3.57	3.79	0.00	010	0.03	***0.000***	4.39	3.25	1.24	0.14	0.76	0.20	***.052***	567 Mus culus antigen, B- receptor gene, plete cds
0114	1.16	1.04	0.96	0.08	0.75	0.18	***0.030***	1.24	1.34	1.29	0.00	0.89	1.30	***.221***	0114 Mouse pa-casein mRNA, plete cds
6395	1.56	1.38	1.58	0.00	0.00	0.48	***0.001***	1.83	1.18	1.03	0.10	0.91	0.97	***.137***	6395 Mouse a-fetoprotein P) gene
6762	4.79	2.65	4.76	0.00	1.37	0.00	***0.093***	0.00	0.72	4.12	1.28	0.00	0.00	***.425***	use interleukin 2) gene, exon 4
2740	1.19	1.33	1 .36	0.79	0.43	0.15	***0.012***	1.21	0.99	1.46	0.00	1.01	0.34	***.077***	2740 Mouse rotropin beta-

															unit (TSH-beta) e
3501	5.31	8.25	3.49	0.00	0.00	0.00	***0.015***	2.02	0.15	6.56	0.00	1.85	0.00	***.182***	3501 Mus musculus secreted T protein (P500/TCA3; SIS-epsilon) NA, complete cds
8449	1.03	0.98	1 .20	0.00	0.00	0.06	***0.000***	1.07	1.16	0.95	1.02	0.44	1.06	***.356***	8449 Mouse Hox- protein mRNA, nd
9015	1.49	1.14	1.04	0.69	0.39	0.09	***0.019***	1.17	1.44	1.29	0.75	0.81	0.96	***.010***	9015 Mouse somal protein L7 7) gene, plete cds
9395	0.76	1.38	2.00	0.00	0.00	0.00	***0.018***	1.63	1.09	2.66	0.08.	1.08	0.92	***.119***	***9395*** Mouse tidine-5'- ophosphate arboxylase NA, 3' end
0441	1.46	1.02	1.17	0.74	0.08	0.50	***0.030***	1.08	1.48	0.98	0.39	1.22	0.00	***.172***	0441 Mouse onless potassium nnel gene MK3
0644	1.30	1.55	1.52	0.87	0.42	0.61	***0.005***	1.47	1.05	1.46	0.32	0.79	0.95	***.052***	***0644*** Mouse is fibroblast wth factor (Fgfb) NA, complete cds
4094	1.09	1.27	2.18	0.00	0.46	0.00	***0.022***	0.45	1.09	1.97	0.00	1 .12	0.91	***.427***	4094 Mouse noic acid- onsive protein) gene, complete
5617	5.22	6.29	1.57	0.00	0.00	0.00	***0.038***	3.23	6.22	0.93	0.00	1.07	0.00	***.119***	***5617*** Mus culus mouse t cell protease-4 NA, complete cds
5875	3.99	3.14	3.34	0.00	0.00	0.00	***0.000***	0.90	3.43	5.14	0.00	1.10	0.38	***.105***	5875 Mouse C class I T3-d e (H-2-d lotype)
1591	1.13	0.78	1.30	0.53	0.59	0.38	***0.025***	1.02	0.98	1.28	0.39	1.70	1.17	***.991***	1591 Murine 0 neutral opeptidase 24.11

															10/NEP) mRNA, ative cds
1243	1.23	1.00	1.00	0.00	0.00	0.59	***0.014***	1.22	0.34	1.65	0.00	1.25	1.41	***.770***	1243 Mouse lens r protein MP70 50) gene, plete cds
3128	1.27	1.33	1.20	0.89	0.00	0.00	***0.032***	1.00	0.77	0.78	1.00	1.32	1.48	***.061***	3128 Mouse eobox protein X2) mRNA, plete cds
989	1.76	0.98	1.42	0.70	0.24	0.00	***0.024***	0.59	0.79	1.16	1 .02	2.00	1.62	***.102***	989 Nkx-5.2 = NK-related eobox gene [mice, E11.5 ryos, mRNA Partial, 1483 nt] TRACTED 3'UTR)
662	1.03	1.38	0.83	0.00	0.21	0.35	***0.009***	1.49	1.29	1.35	1.41	0.67	0.97	***.181***	s musculus ofetal antigen NA, partial cds
982	2.02	1.71	1.86	0.42	0.21	0.28	***0.000***	2.28	2.53	0.98	0.00	1.02	0.00	***.054***	982 Mus culus Balb/c roendothelin-1 e, promoter on
210	5.68	14.95	9.70	0.00	0.00	0.00	***0.012***	6.61	4.29	14.29	0.00	0.00	0.00	***.080***	210 Mus culus oelastin mRNA, plete cds
932	1.42	1.13	1.28	0.86	0.16	0.48	***0.024***	1.40	1.30	0.87	0.00	0.80	1.32	***.313***	932 Mus musculus follicle ulating hormone beta subunit H-beta) gene, complete cds
443	1.24	0.89	1.26	0.00	0.07	0.03	***0.001***	1.11	1.51	1.21	0.13	0.70	1.82	***.480***	443 Mus culus proto- ogene protein c- (c-ros) mRNA, plete cds
252	0.92	1 .07	1 .19	0.00	0.20	0.32	***0.002***	1.19	1.05	1.34	0.95	1.09	0.73	***.114***	252 Mus culus abotropic amate receptor 8 luR8) mRNA,

														plete cds
513	1.01	1.45	1.29	0.68	0.18	0.72	***0.029***	1.09	1.29	1.12	0.00	0.99	0.12	***.067***	513 Mus culus KRAB-Zinc er protein 79 79) mRNA, ial cds
005	3.41	10.25	4.71	0.00	0.00	0.00	***0.043***	0.00	7.73	9.70	7.06.	0.00	0.00	***.412***	s musculus tbc1 NA, complete cds
245	0.89	1.28	1.19	0.00	0.00	0.00	***0.001***	1.21	1.16	1.11	0.00	1.24	0.00	***.145***	245 Mus culus fos-related igen-1 (Fra-1) NA, complete cds
353	1.01	1.29	1.30	0.00	0.49	0.00	***0.005***	1.07	0.99	1.36	0.00	1.07	0.12	***.105***	353 Mus musculus protein sphatase 2A B'alpha3 regulatory unit mRNA, partial cds
189	1.1 1	0.90	0.86	0.20	0.00	0.65	***0.030***	1.40	1.23	1.29	1.10	0.83	1.74	***.777***	189 Mus musculus pancreatic peptide/neuropeptide Y/peptide receptor gene, complete cds
650	1.29	1.15	1.17	0.17	0.64	0.00	***0.009***	1.28	1.30	1.17	0.75	0.85	0.35	***.020***	650 Mus musculus neurexophilin xph-2) gene, large exon and 3' of the intron, and partial cds
513	0.81	1.09	1.05	0.51	0.36	0.59	***0.010***	1.02	1.00	1.00	1.23	0.99	1.35	***.150***	513 Mus musculus Rho- ociated, coiled-coil forming protein se p160 ROCK-2 mRNA, complete
418	0.96	1.45	1.72	0.00	0.78	0.44	***0.037***	2.23	1.04	1.13	1.04	0.51	0.00	***.123***	418 Mus culus Netrin-1 rin-1) mRNA, plete cds
137	1.82	1.59	1.58	0.00	0.72	0.95	***0.020***	1.73	1.03	1.35	0.36	0.97	0.65	***.057***	137 Mus culus T2- herin mRNA, ial cds
208	1.92	19.41	5.95	0.00	0.00	0.00	***0.007***	8.28	13.48	38.84	0.00	0.00	0.00	***.099***	208 Mus culus neurogenin gn3) gene, plete cds

700	5.11	3.18	3.26	0.00	0.00	0.00	***0.004***	5.33	1.30	4.75	0.70	0.00	0.36	***.054***	s musculus serine teinase inhibitor 6 16) mRNA, plete cds.
724	8.41	5.29	5.43	0.00	2.24	0.00	***0.011***	1.15	1.53	0.00	0.85	0.00	0.00	***.324***	s musculus ative sphoinositide 5- sphatase type II NA, complete
755	2.04	2.40	1.68	0.82	1.17	0.10	***0.023***	0.83	1.63	1.18	0.00	0.82	0.71	***.110***	senger RNA ment for mouse rferon beta (type oding for the c- inal part.
479	1.64	1.70	1.36	0.32	0:08	1.17	***0.039***	1.03	0.58	1 .19	0.67	0.97	0.00	***.317***	use mRNA ment for serum loid A (SAA) 3 tein.
123	1.69	1.82	1.59	0.43	0.37	0.99	***0.006***	1.01	1.70	2.45	0.04	0.99	0.56	***.075***	use mRNA for inal xynucleotidyltran ase (TdT).
260	1.15	1.82	2.55	0.00	0.00	0.00	***0.011***	3.64	1.63	0.95	1.05	0.00	0.21	***.130***	use Y mosome RNA script expressed estis (pYMT2/B).
640	1.17	27.06	1.14	0.00	0.00	0.00	***0.000***	2.21	3.04	6.96	0.00	0.00	0.00	***.050***	640 Mouse NF- ene for middle- ecular-mass rofilament protein
115	1.83	1.63	1.23	0.00	0.00	0.17	***0.001***	1.15	1.02	0.98	0.15	0.24	1.02	***.105***	use mRNA for E- herin (= morulin, = L- , = cell-CAM /80, = Arc-1).
368	3.83	11.36	2.49	0.00	0.00	0.00	***0.016***	0.00	0.05	16.10	27.29	1.95	0.00	***.693***	rine mRNA for c- proto-oncogene.
540	1.07	1.23	1.56	0.34	0.00	0.47	***0.007***	1.08	1.38	1.02	0.38	0.98	0.65	***.078***	540 Mouse c-abl e exon 1 of type

														RNA
538	7.03	5.05	0.26	0.00	0.00	0.00	***0.036***	0.00	1.26	14.07	26..77	0.00	0.74	***.702***	rine mRNA for -1.4. protein.
664	1.47	2.17	1.59	0.13	0.76	0.90	***0.023***	1.13	1.20	0.25	0.00	0.18	1.10	***.400***	use mRNA for N-protein (exons 1 - art.).
926	1.00	1.00	1.56	0.00	0.00	0.00	***0.003***	1.22	1.11	1.17	1.17	0.00	0.00	***.117***	use mRNA for eticulin.
972	1.63	2.07	1.66	0.55	0.66	0.85	***0.003***	1.99	1.04	1.95	0.79	0.00	0.96	***.067***	use mRNA for a-adaptin (C).
830	1.42	1.63	1.21	0.56	0.00	0.00	***0.005***	1.12	1.07	0.87	0.93	0.23	1.82	***.956***	rine mRNA for roendocrine tein 7B2.
510	4.28	8.23	5.27	0.00	2.36	0.00	***0.023***	6.86	5.28	0.00	0.00	0.00	0.00	***.123***	use mRNA for 3.3 PRI script.
683	4.96	35.96	6.00	0.00	0.00	0.00	***0.001***	1.44	32.38	0.00	0.00	0.00	0.00	***.136***	usculus T NA.
991	2.51	3.70	4.67	0.00	0.00	0.00	***0.004***	2.85	9.53	3.06	0.00	0.00	0.00	***.079***	991 Mouse NA for homologue he rat T cell erentiation marker
424	2.42	0.95	2.24	0.00	0.00	0.00	***0.016***	1.05	2.97	2.58	0.00	0.35	1.76	***.134***	424 Mouse NA for gamma ptin
315	1.56	5.39	4.68	0.00	0.33	0.44	***0.038***	5.99	1.85	1.68	0.00	0.00	0.00	***.088***	usculus mRNA CAAT-box DNA ing protein unit A (NF-YA) tial).
781	0.99	1.53	1.53	0.06	0.49	0.78	***0.030***	1.10	1.36	1.13	0.72	1.01	0.72	***.040***	781 M.musCUIUS 2 gene
044	1.26	1.03	1.66	0.71	0.40	0.27	***0.019***	1.10	1.23	1.59	0.97	0.47	0.79	***.053***	044 M.musculus NA for protein C
349	2.20	1.22	2.82	0.00	0.78	0.00	***0.027***	6.12	2.09	1.98	0.00	.0.00	0.00	***.067***	349 M.musculus NA for transferrin ptor
960	1.01	56.29	4.13	0.00	0.00	0.00	***0.000***	6.85	17.60	0.00	0.00.	0.00	0.00	***.191***	usculus mRNA

876	0.96	1.54	0.93	0.00	0.00	0.17	***0.006***	2.15	1.64	1.04	3.04	0.00	1.10	***.823***	ribosomal protein 876 Murine
940	1.01	0.88	0.80	0.43	0.24	0.19	***0.003***	1.03	1.55	1.66	0.99	1.15	1.06	***.160***	2 mRNA for . 2 protein use mRNA for a wth.factor- cible immediate y gene (3CH134).
285	2.49	1.96	1.92	0.00	0.00	.0.00	***0.000***	1.64	2.00	0.82	0.00	1.18	0.10	***.108***	285 M.musculus for HC1 locus
449	1.44	1.75	1.43	0.00	0.00	0.00	***0.000***	1.58	1.36	0.64	0.00	0.00	2.12	***.558***	449 M.musculus NA for calcyclin
783	4.50	3.48	4.48	0.47	0.00	1.53	***0.003***	3.31	3.70	0.00	0.00	0.00	0.00	***.117***	usculus VCAM-1 NA.
061	1.34	1.51	1.45	0.74	0.41	0.56	***0.001***	1.55	1.08	1.02	0.98	0.29	0.65	***.093***	usculus HCNGP NA.
620	4.60	22.94	3.86	0.00	0.00	0.00	***0.000***	6.71	34.28	0.00	0.00	0.00	0.00	***.161***	usculus mRNA inhibin beta-B unit.
018	1.41	1.13	1.17	0.29	0.53	0.87	***0.024***	1.27	1.39	0.72	0.85	0.75	2.28	***.775***	018 M.musculus NA for ld4 helix- -helix protein
295	0.99	1.24	1.01	0.00	0.24	0.65	***0.019***	1.17	0.69	1.28	0.58	1.57	1.06	***.939***	295 Mmusculus e for anocortin 5 ptor
557	1.74	2.37	1.37	0.96	0.00	0.00	***0.026***	0.00	1.04	3.07	1.06	0.00	0.00	***.352***	usculus cadL NA.
304	1.82	1.67	0.75	0.66	0.00	0.00	***0.040***	0.08	1.25	1.57	1.25	1.36	0.00	***.889***	304 M. culus (SRP9) al recognition icle subunit
339	1.68	1.68	1.68	0.00	0.31	0.70.	***0.003***	2.01	1.98	0.00	0.29	0.19	1.30	***.382***	NA, 689bp usculus Six1 NA.
320	1.32	1.81	1.76	0.73	0.00	0.39	***0.009***	1.15	1.58	0.85	0.51	0.33	1.70	***.507***	usculus KIS NA.
577	1.23	1.01	0.81	0.48	0.28	0.75	***0.049***	1.12	1.06	1.33	2.09	0.99	0.93	***.685***	usculus mRNA K-glypican.

601	1.16	1.37	0.91	0.00	0.20	0.42	***0.006***	1.21	1.25	1.20	0.22	0.74	1.09	***.101***	usculus PTX3
932	1.54	1.19	1.07	0.66	0.34	0.38	***0.010***	2.12	0.93	1.88	1.12	0.64	0.33	***.092***	NA. usculus mRNA
368	5.46	35.26	0.09	0.00	0.00	0.00	***0.007***	0.00	36.57	54.33	0.00	0.00	0.00	***.131***	ryanodine ptor type 1. usculus putative
352	1.19	1 .26	1 .17	0.10	0.35	0.32	***0.000***	1.47	1.05	1.24	0.64	0.28	0.95	***.053***	scription factor. usculus mRNA
664	1.32	1.29	1.09	0.82	0.70	0.17	***0.033***	1.51	1.46	0.91	0.00	0.42	1.10	***.103***	Bpx protein. 664 M.musculus
606	1.33	1.60	1.31	0.00	0.00	0.00	***0.000***	1.03	1.20	2.07	0.97	0.82	0.00	***.131***	NA for ubiquitin-jugating enzyme M2 usculus mRNA
719	1.64	1.19	1.15	0.59	0.00	0.66	***0.025***	1.98	0.98	1.47	0.51	0.00	1.02	***.079***	Ott protein, clone 7. 719 M.musculus
817	1.16	1.42	1.57	0.55	0.00	0.27	***0.005***	1.39	1.65	0.96	1.04	0.00	0.18	***.071***	ovirus restriction e Fv 1 817 M.nluscLJIUS
581	1.13	0.64	1.18	0.10	0.00	0.14	***0.007***	1.09	1 .15	1.36	0.91	0.00	1.65	***.511***	NA for aphorin F 581 M.musculus gene encoding
500	1.36	1 .92	1.10	0.94	0.34	0.21	***0.044***	0.79	1.06	0.63	0.53	1.13	5.00	***.379***	ocyte-dervied seven smembrane domain receptor, in B6 use glandular
848	6.67	4.44	5.83	0.00	0.00	0.03	***0.001***	2.48	0.00	2.96	1.25	0.75	0.00	***.310***	ikrein gene. use int-2 gene.
224	0.91	1.18	1.37	0.00	0.14	0.53	***0.011***	1.18	1.28	0.97	0.17	1.15	1.03	***.330***	usculus rnRNA
103	0.95	1.40	1.05	0.24	0.19	0.28	***0.003***	1.07	1.16	1.18	0.54	1.08	0.61	***.085***	5HT1E beta tonin receptor. usculus Mox-1
143	1.19	1.38	1.28	0.15	0.67	0.54	***0.007***	1.20	1.71	0.93	0.01	0.16	1.07	***.099***	NA. usculus ALK-6
532	1.23	1.37	1 .27	0.00	0.00	0.00	***0.000***	1.11	1.29	0.65	1.10	0.90	0.00	***.418***	NA, complete usculus mRNA
166	0.95	1.35	1.34	0.00	0.00	0.26	***0.002***	1.71	1.51	1.05	2.29	0.00	0.56	***.544***	follistatin. 166 M.musculus

168	1.00	1.35	1.05	0.05	0.82	0.06	***0.041***	1.26	1.68	1.00	0.55	1.12	0.77	***.128***
147	1.42	1.63	1.08	0.00	0.07	0.00	***0.001***	0.98	0.66	1.18	1.17	0.04	1.02	***.639***

lb/c) mRNA for
agen IV alpha 3
in
168 M.rnusculus
NA for collagen
lpha 5 chain
147 M.musculus
e for cell
esion regulator